VOLUME 45 JUNE, 1955 NUMBER 6

UNIVERSITY OF ILLINOIS
JUN 28 1955
SCIENCE LIBRARY

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONTENTS

Chemical names for active ingredients of fungicides
S. E. A. McCallan, Lawrence P. Miller, and Mary A. Magill 295

Further studies on the nature of Fusarium resistance in tomato
S. S. Gothoskar, R. P. Scheffer, M. A. Stahmann, and J. C. Walker 303

Strawberry vein banding virus ..... Norman W. Frazier 307

Immunity to virus X in potato: Studies of clonal lines
Roland G. Timian, W. J. Hooker, and C. E. Peterson 313

Prunus tomentosa as an index plant for sour cherry viruses ..... Harry C. Fink 320

A peach disorder possibly related to the virus bud failure disease of almond
E. E. Wilson and H. Keith Wagnon 323

Hosts, transmission, and electron microscopy of Cymbidium mosaic virus with special reference to Cattleya leaf necrosis ..... D. D. Jensen and A. H. Gold 327

Biophysical characteristics of the tomato-ringspot virus
Robert P. Kahn, Paul R. Desjardins, and C. A. Senseney 334

Foliage as a source of secondary inoculum for tomato anthracnose
Maria E. Pantidou and W. T. Schroeder 338

Report and abstracts of the 1955 annual meeting of the Southern Division of the American Phytopathological Society 346

News

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

Rohm & Haas **fungicides** available for research

The Rohm & Haas Company, manufacturers of DITHANE and many other outstanding agricultural chemicals, is happy to offer phytopathologists and other workers in the field, test quantities of many different products for experimental use.

Principal Rohm & Haas agricultural fungicides are:

KARATHANE WD—a 25% active ingredient concentrate, suitable for spraying or preparation of dusts, based on dinitro 1-methyl heptyl phenyl crotonate. KARATHANE is highly effective as an eradicant and protectant against representative species of every genus of powdery mildew on fruits, vegetables and ornamentals. An active miticide, it is particularly effective on brown almond mite. Possessing some activity against a variety of diseases, insects and mites, it merits broad consideration in your research work.

DITHANE Z-78 (zineb)—a 65% ethylene bisdithiocarbamate powder suitable for spraying or dust blending. A fungicide, it controls many diseases on a wide variety of crops. Its latest field of application, and one which deserves further study, is in the control of soil fungi responsible for damping-off and "sore-shin" on cotton seedlings.

DITHANE M-22 (maneb)—70% manganese ethylene bisdithiocarbamate. A powder suitable for spraying or dust blending, it is particularly effective in the control of tomato diseases.

DITHANE D-14 (nabam)—19% sodium ethylene bisdithiocarbamate. A water-soluble liquid usually combined with a soluble metallic salt—zinc sulfate or ferric sulfate or manganese sulfate for the preparation of tank-mix fungicides. One development with this product creating considerable interest, is its application as a fungicide when mixed with complex salts of zinc, manganese and iron.

These and many other Rohm & Haas products for use in agriculture have found a wide market. Others are in the developmental stage.

Research workers who would like to have further information or adequate samples of these chemicals for testing purposes are invited to write directly to our main office.

CHEMICALS FOR AGRICULTURE

ROHM & HAAS COMPANY

WASHINGTON SQUARE, PHILADELPHIA 5, PA.

Representatives in principal foreign countries

DITHANE and KARATHANE are trademarks, Reg. U. S. Pat. Off. and in principal foreign countries.

PHYTOPATHOLOGY

An International Journal

Official Organ of The American Phytopathological Society

EDITORS

A. F. Ross, Editor-in-Chief, Cornell University, Ithaca, New York

RALPH M. CALDWELL, Purdue University, Lafayette, Indiana

C. S. HOLTON, State College of Washington, Pullman, Washington

S. E. A. MCCALLAN, Boyce Thompson Institute, Yonkers 3, N. Y.

ASSOCIATE EDITORS

VINCENT W. COCHRANE, Wesleyan University, Middletown, Connecticut

EARLE W. HANSEN, University of Wisconsin, Madison, Wisconsin

M. T. HILBORN, Agricultural Experiment Station, Orono, Maine

W. J. HOOKER, Michigan State College, East Lansing, Michigan

W. F. JEFFERS, College Heights, Hyattsville, Maryland

ARTHUR KELMAN, North Carolina State College, Raleigh, North Carolina

FRANK P. MCWHORTER, Oregon State College, Corvallis, Oregon

EDWARD O. OLSON, Citrus Rootstock Investigations, Box 144, Weslaco, Texas

MORTIMER P. STARR, University of California, Davis, California

ALBERT L. TAYLOR, Plant Industry Station, Beltsville, Maryland

R. P. TRUE, West Virginia University, Morgantown, West Virginia

C. E. YARWOOD, University of California, Berkeley, California

BUSINESS MANAGEMENT

SAUL RICH, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

L. J. ALEXANDER, Advertising Manager, Agricultural Experiment Station, Wooster, Ohio.

NOTICES

Send communications regarding subscriptions and back numbers to SAUL RICH, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

Subscription price. $10.00 per year, domestic and foreign. Single current numbers (within 60 days of publication), $1.00. The journal has been issued monthly beginning with Volume 8. **Separate Numbers** may be purchased from back volumes at $1.00 per copy. Complete unbound volumes, 1 through 7, when available, $6.00 each; 8 through 41, as available, $10.00 each. **Thirty-Year Index** (Vols. 1–30, 1911–1940), bound: $7.50, domestic and foreign. **Ten-Year Index** (Vols. 31–40, 1941–1950), paper cover, $3.50. **Lost Numbers.** Claims for copies (domestic subscriptions) lost in the mails must be received within 60 days from the date of issue (Jan. 15 and monthly thereafter); otherwise the usual charge of $1.00 will be made for replacements. Deliveries overseas are not guaranteed and foreign mailings are made at the subscriber's risk. **Separates.** No gratis copies supplied. Quotation is sent with proof.

Advertisements. Rates may be secured from the Advertising Manager. No indorsement of any statement of claims made in advertisements is assumed by this Journal or by The American Phytopathological Society.

Manuscripts and editorial correspondence should be sent to the Editor-in-Chief. At least 1 of the authors must be a member of the American Phytopathological Society. Type on only 1 side of sheet; double-space all parts of manuscript, including footnotes, citations, and legends. For papers longer than 1 printed page, the article should begin with a brief, concise summary. Number footnotes consecutively with arabic numerals, except in tables where lower case letters are used. Give each table a heading stating its contents. Make references to literature as footnotes only when 5 or fewer citations are given. Name and address of the institution where the work was done should precede "Literature Cited." List references alphabetically in "Literature Cited" and number consecutively throughout text as well as in list of citations. Authors are advised to read "Preparing manuscripts for Phytopathology" by W. C. Price, published in PHYTOPATHOLOGY 44: 667-674, 1954, as well as current issues of PHYTOPATHOLOGY.

Illustrations must be limited in number and should be sharp and clear. One-half the cost of engraving in excess of $16.00, the approximate cost of a single full page cut or of two 3 in. x 4½ in. cuts, are charged to the author. No charge is made for line drawings that substitute for tables unless drawings and tables together exceed 30 per cent of the length of the article. Illustrations are run as text figures designated by arabic numerals. Units of a composite illustration are designated by letters. Arrange full-page figures to conform to journal proportions (6⅛ x 8½ in.), making due allowance for reductions. Specific reference to each illustration must be made in the text, and each must be accompanied by an adequate legend, typed double-space on a separate paper and not on the illustration.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

FOUNDED 1908. INCORPORATED 1915.

THE A. P. S. COUNCIL

President—James H. Jensen, Provost, Iowa State College, Ames, Iowa
Vice-President—George W. Fischer, Washington State College, Pullman, Washington
Secretary—Glenn S. Pound, University of Wisconsin, Madison 6, Wisconsin
Treasurer—Saul Rich, Connecticut Agricultural Experiment Station, P. O. Drawer 1106, New Haven 4, Connecticut
Editor-in-Chief—A. F. Ross, Cornell University, Ithaca, New York
President-Elect—Helen Hart, University of Minnesota, St. Paul 1, Minnesota
Retiring President—George F. Weber, University of Florida, Gainesville, Florida
Councilors:

E. C. Blodgett, Irrigation Experiment Station, Prosser, Washington
J. L. Allison, North Carolina State College, Raleigh, North Carolina
Paul R. Miller, Plant Disease Survey, U. S. Department of Agriculture, Beltsville, Maryland
E. G. Rex, New Jersey Department of Agriculture, Trenton, New Jersey
J. R. Shay, Purdue University, Lafayette, Indiana
W. C. Snyder, University of California, Berkeley, California
W. D. McClellan, U. S. Department of Agriculture, Beltsville, Maryland

DIVISIONS OF THE SOCIETY

Northeastern: A. B. Burrell, Cornell University, Ithaca, New York, *Pres.;* A. C. Tarjan, University of Rhode Island, Kingston, Rhode Island, *Vice-Pres.;* B. H. Davis, Rutgers University, New Brunswick, New Jersey, *Sec.-Treas.*

North Central: Donald Cation, Michigan State College, East Lansing, Michigan, *Pres.;* C. O. Johnston, Kansas State College, Manhattan, Kansas, *Vice-Pres.;* M. B. Linn, University of Illinois, Urbana, Illinois, *Sec.-Treas.*

Pacific: George A. Zentmyer, Citrus Experiment Station, Riverside, California, *Pres.;* S. M. Dietz, Oregon State College, Corvallis, Oregon, *Vice-Pres.;* Roy A. Young, Oregon State College, Corvallis, Oregon, *Sec.-Treas.*

Potomac: H. Rex Thomas, Plant Industry Station, Beltsville, Maryland, *Pres.;* J. G. Leach. West Virginia University, Morgantown, West Virginia, *Vice-Pres.;* L. O. Weaver, University of Maryland, College Park, Maryland, *Sec.-Treas.*

Southern: T. W. Graham, Pee Dee Experiment Station, Florence, South Carolina, *Pres.;* J. A. Lyle, Alabama Agricultural Experiment Station, Auburn, Alabama, *Vice-Pres.;* G. B. Lucas, Plant Pathology, North Carolina State College, Raleigh, North Carolina, *Sec.-Treas.*

SUSTAINING ASSOCIATES

American Tobacco Company, Inc., Research Laboratory, 400 Petersburg Turnpike, Richmond 24, Virginia.

The Dow Chemical Company, Midland, Michigan

E. I. du Pont de Nemours & Company, Incorporated, Wilmington 98, Delaware

Ferry-Morse Seed Company, P. O. Box 778, Detroit 31, Michigan

Freeport Sulphur Company, New Orleans 5, Louisiana

H. J. Heinz Company, Pittsburgh 30, Pennsylvania

Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

Mathieson Chemical Corporation, Mathieson Building Baltimore, Maryland

Rohm & Haas Company, Washington Square, Philadelphia 5, Pennsylvania

Shell Chemical Corporation, Julius Hyman & Company Division, P. O. Box 2171, Denver 1, Colorado

Tennessee Corporation, 619-27 Grant Building, Atlanta 1, Georgia

Yoder Brothers, Inc., Barberton, Ohio

CHEMICAL NAMES FOR ACTIVE INGREDIENTS OF FUNGICIDES

S. E. A. McCallan, Lawrence P. Miller, and Mary A. Magill [1]

The list of chemical names for active ingredients of fungicides has been prepared to assist authors of manuscripts for PHYTOPATHOLOGY and others interested in the chemistry of fungicidal action. It is hoped also that the availability of these names will encourage the practice of reporting in scientific publications the complete chemical name of fungicides rather than only the trade-mark or code name, the latter of which, in particular, is without meaning to the great majority of readers. In full length papers, a fungicide should be identified at least once by the chemical name, and also by formula when necessary for proper identification. This procedure should be followed in abstracts except that common names alone may be used. Authors should specify also the percentage of active ingredients.

The system of nomenclature used by the American Chemical Society as exemplified in CHEMICAL ABSTRACTS has been followed. The chemical names should be written exactly as given below, including the punctuation and spelling. Common names, including coined names, are identified by the superscript ° and alternate chemical names by the superscript *. Formulae have been given for the chromate complexes and for a few copper fungicides, since the names are insufficient identification. A few of the trade- or code-named fungicides are known to be no longer generally available, but since they still may be of scientific interest they have been kept on the list and are marked thus †. Some trade-named fungicides are prepared in different formulations or with different percentages of the same active ingredients and are distinguished by different code numbers or letters; these are entered on 1 line separated by semicolons. Some fungicides with different trade names contain the same active ingredients in similar or different percentages; these have been entered separately. When fungicides contain 2 or more active ingredients, the names of the ingredients are separated by semicolons. Some of the seed-treatment mixtures also contain the insecticide lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane).

In general, the list of fungicides, which also includes seed disinfectants, soil fumigants, chemotherapeutants, agricultural bactericides, and wood preservatives, is limited to those of current interest, as indicated by reports during the 5-year period 1950-54, in PHYTOPATHOLOGY, THE PLANT DISEASE REPORTER, and the Annual Results of Tests with Newer Fungicides published in AGRICULTURAL CHEMICALS. In addition the fungicides more frequently mentioned in THE REVIEW OF APPLIED MYCOLOGY during the same period also are given. A few of the fungicides mentioned in this literature could not be adequately identified chemically, and so had to be omitted. No attempt has been made to include all the numerous trade-named formulations on the market.

To aid in checking chemical names of active ingredients without the use of trade or common names, the list of active ingredients is also given in alphabetical order based on the chemical names.

The compilers are indebted to various individuals for helpful suggestions and advice, and to members of the Nomenclature Committee of the American Chemical Society Division of Organic Chemistry and of the American Chemical Society Committee on Nomenclature, Spelling, and Pronunciation for their cooperation. The compilers would appreciate having corrections and fungicides with new active ingredients brought to their attention.

[1] Respectively, Plant Pathologist, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York; Biochemist, Boyce Thompson Institute for Plant Research; and Associate Editor of Chemical Abstracts, Ohio State University, Columbus, Ohio. Dr. Magill is a member of the American Chemical Society Committee on Nomenclature, Spelling, and Pronunciation and of the Nomenclature Committee of the Division of Organic Chemistry of the American Chemical Society.

FUNGICIDE	ACTIVE INGREDIENT
Aagrano 48; Aagrano 250; Aagrano 350	(3-ethoxypropyl) mercury bromide
Acti-dione	see Cycloheximide
Agri-mycin; Agri-mycin 100	see Streptomycin and Oxytetracycline
Agristrep; Agstrep	streptomycin sulfate (see Streptomycin)
Agrosan C	phenylmercury acetate; ethylmercury chloride; mercuric oxide
Agrosan G	tolylmercury acetate; ethylmercury chloride
Agrosan GN	tolylmercury acetate
Agrox	phenylmercuriurea

FUNGICIDE	ACTIVE INGREDIENT
Agrox C	phenylmercury acetate; ethylmercury chloride; mercuric oxide
Amine 220	2-(8-heptadecenyl)-2-imidazoline-1-ethanol
Anticarie	hexachlorobenzene
Arasan; Arasan M; Arasan SF†; Arasan SF-X	see Thiram
Arathane (now Karathane)	2-(1-methylheptyl)-4,6-dinitrophenyl crotonate and isomers
Aretan	2-methoxyethylmercury chloride
AS-15	see Streptomycin
Aureomycin	see Chlortetracycline
Barbak	*N,N'*-mercuridicarbanilonitrile
Basi-Cop	basic copper sulfate
Bioquin	8-quinolinol
Bioquin 1	copper derivative of 8-quinolinol
Bisphenol A	4,4'-isopropylidenediphenol
Blitox	basic copper chloride
Boliden Salt	reaction product of arsenic acid, sodium arsenate, sodium dichromate, and zinc sulfate
Bordeaux mixture[c]	reaction product of copper sulfate and calcium hydroxide
Brassicol	pentachloronitrobenzene
Bunt-cure	hexachlorobenzene
Bunt-no-more	hexachlorobenzene
Cadminate	cadmium succinate
Calo-clor	mercurous chloride; mercuric chloride
Calocure	mercurous chloride; mercuric chloride
Calogreen	mercurous chloride
C & C Experimental Chemotherapeutant 1207	2-norcamphanemethanol
C & C Experimental Fungicide 224	mercury zinc chromate ($0.4HgO{\cdot}3ZnO{\cdot}CrO_3$)
C & C Experimental Fungicide 640	copper zinc chromate ($4CuO{\cdot}ZnO{\cdot}CrO_3{\cdot}xH_2O$)
C & C Experimental Fungicide 1025	copper mercury cadmium zinc calcium chromate ($2.5CuO{\cdot}0.15HgO{\cdot}6CdO{\cdot}ZnO{\cdot}10CaO{\cdot}CrO_3$)
C & C Experimental Fungicide 1182	2-(4-chloro-3,5-xylyloxy)ethanol
C & C Experimental Fungicide 5379†	1,2,3-trithia-5,8-diazacyclononane-4,9-dithione
C & C Experimental Fungicide 5400†	1,1'-trithiobis[*N,N*-dimethylthioformamide]
Captan[c]	*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
Captan 50-W	see Captan
Carbamate	see Ferbam
Carboxide	ethylene oxide
Celcure	reaction product of copper sulfate and sodium dichromate
Ceresan Fungicide; Ceresan M; Ceresan M-2X	*N*-(ethylmercuri)-*p*-toluenesulfonanilide
Ceresan, 2%	ethylmercury chloride
Chemonite	reaction product of cupric hydroxide, arsenic trioxide, acetic acid, and ammonium hydroxide
Chinosol	8-quinolinol sulfate
Chloramphenicol[c]	D(−)-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide
Chloranil[c]	tetrachloro-*p*-benzoquinone
Chloromycetin	see Chloramphenicol
Chloropicrin[c]	trichloronitromethane
Chlortetracycline[c]	7-chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
Clorox	sodium hypochlorite

FUNGICIDE	ACTIVE INGREDIENT
CM 218 Spike Spray	*N*-phenylmercuriethylenediamine; sulfur
C-O-C-S	basic copper chloride; basic copper sulfate
Colsul	sulfur
Copox	cuprous oxide
Copper A Compound	calcium copper chloride ($4Cu(OH)_2 \cdot CaCl_2 \cdot 4H_2O$)
Copper Cupferron	copper derivative of *N*-nitroso-*N*-phenylhydroxylamine
Copper Hydro	basic copper sulfate
Copperized CZC	reaction product of cupric chloride, zinc chloride, and sodium dichromate
Copper Sandoz	cuprous oxide
Corodinoc	sodium 4,6-dinitro-*o*-cresoxide
Coromate	see Ferbam
Coromerc	*N*-phenylmercuriethylenediamine
Corosul D; Corosul S	sulfur
Corozate	see Ziram
CR 305	2,2′-thiobis[4-chlorophenol]
CR 1639	2-(1-methylheptyl)-4,6-dinitrophenyl crotonate and isomers
Crag Fruit Fungicide 341; Crag Fruit Fungicide 341SC†	see Glyodin
Crag Fruit Fungicide 341B†	2-heptadecyl-2-imidazoline; 2-pentadecyl-2-imidazoline; 2-(8-heptadecenyl)-2-imidazoline; 2-heptadecyl-2-imidazoline-1-ethanol
Crag Fruit Fungicide 341C†	2-heptadecyl-2-imidazoline acetate; 2-pentadecyl-2-imidazoline acetate; 2-(8-heptadecenyl)-2-imidazoline acetate
Crag Industrial Fungicide 974	tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione
Crag Potato Fungicide 658	copper zinc chromate ($15CuO \cdot 10ZnO \cdot 6CrO_3 \cdot 25H_2O$)
Crag Turf Fungicide 531	copper cadmium zinc calcium chromate ($2.5CuO \cdot 0.6CdO \cdot ZnO \cdot 10CaO \cdot CrO_3$)
Cunilate 2472	copper derivative of 8-quinolinol
Cuprenox	basic copper chloride
Cuprinol	copper naphthenate
Cuprocide	cuprous oxide
Cupro-K	basic copper chloride
Cuprokylt	basic copper chloride
Cuprosana	basic copper chloride
Cuprox	basic copper chloride
Cycloheximide[c]	3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide
D-D	1,3-dichloropropene; 1,2-dichloropropane
Dehydroacetic acid[c]	2-acetyl-5-hydroxy-3-oxo-4-hexenoic acid δ-lactone
DHA	see Dehydroacetic acid
DHA-S	sodium derivative of 2-acetyl-5-hydroxy-3-oxo-4-hexenoic acid δ-lactone
Dichlone[c]	2,3-dichloro-1,4-naphthoquinone
Dithane D-14	see Nabam
Dithane M-22	see Maneb
Dithane Z-78	see Zineb
DN-111	dicyclohexylamine salt of 2-cyclohexyl-4,6-dinitrophenol
DN-289	triethanolamine salt of 2-*sec*-butyl-4,6-dinitrophenol
Dow 9B	zinc 2,4,5-trichlorophenoxide
Dowfume G†	bromomethane
Dowfume MC-2	bromomethane; trichloronitromethane
Dowfume N†	1,3-dichloropropene; 1,2-dichloropropane
Dowfume W-40; Dowfume W-85	1,2-dibromoethane
Dowicide A	sodium *o*-phenylphenoxide
Dowicide B	sodium 2,4,5-trichlorophenoxide
Dowicide F	sodium 2,3,4,6-tetrachlorophenoxide
Dowicide G	sodium pentachlorophenoxide
Dowicide 1	*o*-phenylphenol
Dowicide 2	2,4,5-trichlorophenol
Dowicide 6	2,3,4,6-tetrachlorophenol
Dowicide 7	pentachlorophenol

FUNGICIDE	ACTIVE INGREDIENT
Dowspray 66 Improved[†]	2-*sec*-butyl-4,6-dinitrophenol
du Pont Liquid 244 Seed Disinfectant; du Pont Liquid 364 Seed Disinfectant	phenylmercury acetate; ethylmercury acetate
Dynacide	*N*-phenylmercuriethylenediamine acetate
Elgetol	sodium 4,6-dinitro-*o*-cresoxide
Emmi	1,4,5,6,7,7-hexachloro-*N*-(ethylmercuri)bicyclo[2.2.1]hept-5-ene-2,3-dicarboximide
Erdalith	reaction product of potassium dichromate, copper sulfate, and arsenic pentoxide
Ethyl B-622	2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine
Ethylene dibromide[a]	1,2-dibromoethane
Ethylmercury perthiocyanate[a]	3,5-bis(ethylmercurithio)-1,2,4-thiadiazole
Ferbam[c]	ferric dimethyldithiocarbamate
Ferberk	see Ferbam
Fermate	see Ferbam
Fernasan	see Thiram
Ferradow	see Ferbam
Fusariol	ethylmercury cyanide
G 4	2,2′-methylenebis[4-chlorophenol]
Gallotox	phenylmercury acetate
Garden Dowfume	1,2-dibromoethane
Geigy SP-50	see Chloranil
Germisan	phenylmercury derivative of pyrocatechol
Glyodin[c]	2-heptadecyl-2-imidazoline acetate
Good-rite p.e.p.s.	polyethylene polysulfide
Good-rite SDD	sodium dimethyldithiocarbamate
Good-rite z.a.c.	cyclohexylamine compound with zinc dimethyldithiocarbamate
Granosan M; Granosan M2-X	*N*-(ethylmercuri)-*p*-toluenesulfonanilide
Granosan, 2%	ethylmercury chloride
Greensalt	reaction product of potassium dichromate, copper sulfate, and arsenic pentoxide
Gytrete	phenylmercury acetate
Half-ounce Leytosan	phenylmercuriurea
I and D Seed Protectant	bis(dimethylthiocarbamoyl) disulfide; γ-1,2,3,4,5,6-hexachlorocyclohexane
Iscobrome	bromomethane
Iscobrome D	1,2-dibromoethane
Iscothan	2-(1-methlyheptyl)-4,6-dinitrophenyl crotonate and isomers
Isothan Q15	2-dodecylisoquinolinium bromide
Isotox-PMA Seed Treater	phenylmercury acetate; γ-1,2,3,4,5,6-hexachlorocyclohexane
J 49	1-(*p*-chlorophenyl)-3,5-dimethyl-4-nitropyrazole
Karathane	2-(1-methylheptyl)-4,6-dinitrophenyl crotonate and isomers
Karbam Black	see Ferbam
Karbam White	see Ziram
KF 467	3,5-bis(ethylmercurithio)-1,2,4-thiadiazole
Kolo 100	sulfur; 2,3-dichloro-1,4-naphthoquinone
Kolofog	sulfur
Kolokarbam	sulfur; ferric dimethyldithiocarbamate
Kolospray	sulfur
Koneprox	basic copper chloride
Krenite	sodium 4,6-dinitro-*o*-cresoxide

FUNGICIDE	ACTIVE INGREDIENT
Larvacide	see Chloropicrin
Leytosan	phenylmercuriurea
Leytosan G91	phenylmercuriurea; γ-1,2,3,4,5,6-hexachlorocyclohexane
Leytosol C	phenylmercuriurea
Lime sulfur[c]	calcium polysulfides
Lunasan	1-(ethylmercuri)-2-thiourea
Lysol	mixture of crude cresols
M-294	copper derivative of *N*-nitroso-*N*-phenylhydroxylamine
Magnetic 70; Magnetic 90; Magnetic 95	sulfur
Malachite green[c]	oxidation product of 4,4′-benzylidenebis[*N*,*N*-dimethylaniline]
Maneb[c]	manganous ethylenebis[dithiocarbamate]
Manzate	see Maneb
Mathieson 275; Mathieson 275D	pentachloronitrobenzene
MEMA	2-methoxyethylmercury acetate
MEML	2-methoxyethylmercury lactate
Merculine; Merculine 100[†]	phenylmercury salicylate
Mercuran; Mercuran AL; Mercuran AS	2-methoxyethylmercury acetate
Mercusan	phenylmercury salicylate; copper salicylate
Mergamma	phenylmercuriurea; γ-1,2,3,4,5,6-hexachlorocyclohexane
Mergamma C	phenylmercury acetate; ethylmercury chloride; γ-1,2,3,4,5,6-hexachlorocyclohexane
Merlane	phenylmercury acetate; ethylmercury chloride; γ-1,2,3,4,5,6-hexachlorocyclohexane
Mersolite 8	phenylmercury acetate
Methasan	see Ziram
Methyl bromide[a]	bromomethane
Microgel	basic copper sulfate
MMDD	cyano(methylmercuri)guanidine
Mycon	thioarsenosomethane
Nabam[c]	disodium ethylenebis[dithiocarbamate]
Natriphene	sodium *o*-phenylphenoxide
New Improved Ceresan	ethylmercury phosphate
New Improved Granosan	ethylmercury phosphate
Nirit	2,4-dinitrophenyl thiocyanate
No-bunt	hexachlorobenzene
Nomersan	see Thiram
Orthocide 406; Orthocide 75; Orthocide 50 Wettable; Orthocide 5% Dust	see Captan
Orthocide Seed Protectant; Ortho Seed Guard	*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide; γ-1,2,3,4,5,6-hexachlorocyclohexane
OS-1199[†]	*trans*-1,4-dibromo-2-butene
Ovex Dust; Ovotran Wettable	*p*-chlorophenyl *p*-chlorobenzenesulfonate
Oxine	8-quinolinol
Oxy Quin	8-quinolinol benzoate
Oxytetracycline[c]	4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
Panogen	cyano(methylmercuri)guanidine
Parzate	see Zineb
Parzate Liquid	see Nabam
PCNB	pentachloronitrobenzene
Pennsalt NP 1083	1-fluoro-2,4-dinitrobenzene

FUNGICIDE	ACTIVE INGREDIENT
Pentrete	phenylmercury acetate
Perenox	cuprous oxide
Permacide	pentachlorophenol
Phix	phenylmercury acetate
Phygon; Phygon XL	see Dichlone
Plant Fungi Control	pentachloronitrobenzene
PMA; PMAC; PMAS	phenylmercury acetate
Pomasol (formerly Pomarsol)	see Thiram
Preventol G-D	2,2′-methylenebis[4-chlorophenol]
Pulpasan F; Pulpasan FF; Pulpasan KP	phenylmercury acetate
Puraseed	*N*-phenylmercuriformamide; anilinocadmium lactate
Puratized Agricultural Spray; Puratized N5E	tris(2-hydroxyethyl)(phenylmercuri)ammonium lactate
Puratized Apple Spray	2-(phenylmercuriamino)ethanol acetate (salt)
Puratized C4-10	phenylmercury lactate
Puratized C13-1212	*N*-phenylmercuriformamide; anilinocadmium lactate
Puratized 806	*N*-phenylmercuriformamide
Puraturf 177	anilinocadmium lactate
Purex	sodium hypochlorite
Quenate Solution 10%	8-quinolinol benzoate
Quinosol	8-quinolinol sulfate
Robertson's Fungicide	cuprous oxide
Sanocide Wheat Seed Protectant	hexachlorobenzene
Santobrite	sodium pentachlorophenoxide
Seedox	2,4,5-trichlorophenyl acetate
Semesan	2-chloro-4-(hydroxymercuri)phenol
Semesan Bel	2-chloro-4-(hydroxymercuri)phenol and isomers; 4-(hydroxymercuri)-2-nitrophenol and isomers
Semesan Jr.†	ethylmercury phosphate
Setrete	phenylmercury acetate
Shell 162; Shell P162	hexachloro-1,3-cyclopentadiene
Shell Copper Fungicide	basic copper chloride
Shirlan; Shirlan AG	salicylanilide
Soilfume 80-20	1,2-dibromoethane
Special Semesan	2-chloro-4-(hydroxymercuri)phenol and isomers; 4-(hydroxymercuri)-*o*-cresol and isomers
Spergon	see Chloranil
Spersul	sulfur
Spraycop	basic copper sulfate
SR 406	see Captan
Stauffer N-244	3-(*p*-chlorophenyl)-5-methylrhodanine
Stauffer N-521	tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione
Stauffer N-869	sodium methyldithiocarbamate
Streptomycin	no chemical name in use
Sulforon; Sulforon X	sulfur
Tag; Tag 331; Tag HL 331	phenylmercury acetate
Tat-C-Lect	phenylmercury acetate
Terramycin	see Oxytetracycline
Tersan†; Tersan 75	see Thiram
Tetramethylthiuram disulfide[a]	bis(dimethylthiocarbamoyl) disulfide
Thiovit	sulfur
Thiram[c]	bis(dimethylthiocarbamoyl) disulfide
TMTD	see Thiram

FUNGICIDE	ACTIVE INGREDIENT
Tri-Basic	basic copper sulfate ($CuSO_4 \cdot 3Cu(OH)_2$)
Tritisan	pentachloronitrobenzene
Tuads	see Thiram
Tulisan	see Thiram
USR-36L	*p*-(3,5-dimethyl-4-nitroso-1-pyrazolyl) benzenesulfonamide
Vancide 51	sodium dimethyldithiocarbamate; sodium derivative of 2-mercaptobenzothiazole
Vancide 51ZW†; Vancide Z-65	zinc dimethyldithiocarbamate; zinc derivative of 2-mercaptobenzothiazole
Velsicol 50-CS-46	1,4,5,6,7,7-hexachloro-*N*-(ethylmercuri) bicyclo[2.2.1]hept-5-ene-2,3-dicarboximide
Verisan	2-methoxyethylmercury silicate
Wood-Ridge Mixture 21	mercurous chloride; mercuric chloride
XP-47†	*trans*-1,4-dibromo-2-butene
Yellow Cuprocide	cuprous oxide
Zerlate	see Ziram
Zinc Coposil	basic cupric zinc sulfate (Cu 19%, Zn 19%)
Zineb®	zinc ethylenebis[dithiocarbamate]
Ziram®	zinc dimethyldithiocarbamate
Zirberk	see Ziram

CHEMICAL NAMES

2-Acetyl-5-hydroxy-3-oxo-4-hexenoic acid δ-lactone
2-Acetyl-5-hydroxy-3-oxo-4-hexenoic acid δ-lactone, sodium derivative of
Anilinocadmium lactate

Basic copper chloride
Basic copper sulfate
Basic cupric zinc sulfate
Bis(dimethylthiocarbamoyl) disulfide
3,5-Bis(ethylmercurithio)-1,2,4-thiadiazole
Bromomethane
2-*sec*-Butyl-4,6-dinitrophenol
2-*sec*-Butyl-4,6-dinitrophenol, triethanolamine salt of

Cadmium succinate
Calcium copper chloride
Calcium polysulfides
7-Chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
2-Chloro-4-(hydroxymercuri) phenol
p-Chlorophenyl *p*-chlorobenzenesulfonate
1-(*p*-Chlorophenyl)-3,5-dimethyl-4-nitropyrazole
3-(*p*-Chlorophenyl)-5-methylrhodanine
2-(4-Chloro-3,5-xylyloxy) ethanol
Copper cadmium zinc calcium chromate
Copper derivative of *N*-nitroso-*N*-phenylhydroxylamine
Copper derivative of 8-quinolinol
Copper mercury cadmium zinc calcium chromate
Copper naphthenate
Copper salicylate
Copper zinc chromate
Cuprous oxide
Cyano(methylmercuri) guanidine
Cyclohexylamine compound with zinc dimethyldithiocarbamate
2-Cyclohexyl-4,6-dinitrophenol, dicyclohexylamine salt of

trans-1,4-Dibromo-2-butene
1,2-Dibromoethane
2,4-Dichloro-6-(*o*-chloroanilino)-*s*-triazine
D(−)-*threo*-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide
2,3-Dichloro-1,4-naphthoquinone
1,2-Dichloropropane
1,3-Dichloropropene
Dicyclohexylamine salt of 2-cylcohexyl-4,6-dinitrophenol
4-Dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
p-(3,5-Dimethyl-4-nitroso-1-pyrazolyl) benzenesulfonamide
3-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide
2,4-Dinitrophenyl thiocyanate
Disodium ethylenebis[dithiocarbamate]

2-Dodecylisoquinolinium bromide

(3-Ethoxypropyl)mercury bromide
Ethylene dibromide[a]
Ethylene oxide
1-(Ethylmercuri)-2-thiourea
N-(Ethylmercuri)-*p*-toluenesulfonanilide
Ethylmercury acetate
Ethylmercury chloride
Ethylmercury cyanide
Ethylmercury phosphate

Ferric dimethyldithiocarbamate
1-Fluoro-2,4-dinitrobenzene

2-(8-Heptadecenyl)-2-imidazoline
2-(8-Heptadecenyl)-2-imidazoline acetate
2-(8-Heptadecenyl)-2-imidazoline-1-ethanol
2-Heptadecyl-2-imidazoline
2-Heptadecyl-2-imidazoline acetate
2-Heptadecyl-2-imidazoline-1-ethanol
Hexachlorobenzene
γ-1,2,3,4,5,6-Hexachlorocyclohexane
Hexachloro-1,3-cyclopentadiene
1,4,5,6,7,7-Hexachloro-*N*-(ethylmercuri)bicyclo[2.2.1]hept-5-ene-2,3-dicarboximide
4-(Hydroxymercuri)-*o*-cresol
4-(Hydroxymercuri)-2-nitrophenol

4,4′-Isopropylidenediphenol

Manganous ethylenebis[dithiocarbamate]
2-Mercaptobenzothiazole
2-Mercaptobenzothiazole, sodium derivative of
2-Mercaptobenzothiazole, zinc derivative of
Mercuric chloride
Mercuric oxide
N,*N*′-Mercuridicarbanilonitrile
Mercurous chloride
Mercury zinc chromate
2-Methoxyethylmercury acetate
2-Methoxyethylmercury chloride
2-Methoxyethylmercury lactate
2-Methoxyethylmercury silicate
Methyl bromide[a]
2,2′-Methylenebis[4-chlorophenol]
2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate

N-Nitroso-*N*-phenylhydroxylamine, copper derivative of
2-Norcamphanemethanol

Pentachloronitrobenzene
Pentachlorophenol
2-Pentadecyl-2-imidazoline
2-Pentadecyl-2-imidazoline acetate
2-(Phenylmercuriamino)ethanol acetate (salt)
N-Phenylmercuriethylenediamine
N-Phenylmercuriethylenediamine acetate
N-Phenylmercuriformamide
Phenylmercuriurea
Phenylmercury acetate
Phenylmercury derivative of pyrocatechol
Phenylmercury lactate
Phenylmercury salicylate
o-Phenylphenol
Polyethylene polysulfide
Pyrocatechol, phenylmercury derivative of

8-Quinolinol
8-Quinolinol benzoate
8-Quinolinol, copper derivative of
8-Quinolinol sulfate

Salicylanilide
Sodium derivative of 2-acetyl-5-hydroxy-3-oxo-4-hexenoic acid δ-lactone
Sodium derivative of 2-mercaptobenzothiazole
Sodium dimethyldithiocarbamate
Sodium 4,6-dinitro-*o*-cresoxide
Sodium hypochlorite
Sodium methyldithiocarbamate
Sodium pentachlorophenoxide
Sodium *o*-phenylphenoxide
Sodium 2,3,4,6-tetrachlorophenoxide
Sodium 2,4,5-trichlorophenoxide
Sulfur

Tetrachloro-*p*-benzoquinone
2,3,4,6-Tetrachlorophenol
Tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione
Tetramethylthiuram disulfide[a]
Thioarsenosomethane
2,2′-Thiobis[4-chlorophenol]
Tolylmercury acetate
N-(Trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
Trichloronitromethane
2,4,5-Trichlorophenol
2,4,5-Trichlorophenyl acetate
Triethanolamine salt of 2-*sec*-butyl-4,6-dinitrophenol
Tris(2-hydroxyethyl)(phenylmercuri)ammonium lactate
1,2,3-Trithia-5,8-diazacyclononane-4,9-dithione
1,1′-Trithiobis[*N*,*N*-dimethylthioformamide]

Zinc derivative of 2-mercaptobenzothiazole
Zinc dimethyldithiocarbamate
Zinc dimethyldithiocarbamate, cyclohexylamine compound with
Zinc ethylenebis[dithiocarbamate]
Zinc 2,4,5-trichlorophenoxide

FURTHER STUDIES ON THE NATURE OF FUSARIUM RESISTANCE IN TOMATO [1]

S. S. Gothoskar, R. P. Scheffer, M. A. Stahmann, and J. C. Walker

SUMMARY

Extracts of single-gene-resistant tomato plants (var. Jefferson) were not inhibitory to the growth of *Fusarium lycopersici* (Sacc.) Snyder & Hansen. Both Jefferson (resistant) and Bonny Best (susceptible) cuttings when treated with culture filtrates and with commercial pectinase preparation developed similar disease symptoms. Resistance then could not readily be attributed to some mechanism of detoxification found in resistant plants but not in susceptible plants. Treatment of Jefferson cuttings with the respiratory inhibitors 2,4-dinitrophenol, thiourea, sodium fluoride, and sodium diethyl dithiocarbamate broke down resistance. These inhibitors had no stimulating effect on growth of the parasite *in vitro*. It is postulated, therefore, that resistance in the tomato plant is closely linked with the metabolism of the plant and is probably due to a very labile substance continuously formed at the expense of energy obtained from respiratory processes.

In a recent paper Scheffer and Walker (11) reported upon a study of the nature and distribution of resistance in tomato to wilt incited by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen. When cuttings of single-gene resistant plants and susceptible plants were placed in suspensions of bud-cells of the fungus, the latter were distributed throughout the vascular system in 5–9 hours. The cuttings were trimmed at the cut end, placed in mineral nutrient solution until roots formed, and then potted in sand and placed in the greenhouse under conditions favorable for disease development. Disease symptoms appeared in both resistant and susceptible plants. The resistant varieties, however, generally showed less severe symptoms than did susceptible ones, invariably recovered, and grew normally after the initial disease shock. Susceptible varieties became progressively more diseased. When different parts of plants were assayed during midwinter conditions in the greenhouse by plating for presence of the fungus, the fungus was recovered after 35 days from the bases of stems of both resistant and susceptible plants. In resistant plants, however, it was not recovered after 20 days from a point midway between base and top of the stem and not after 10 days from the top of the stem. When plants were shaded and therefore were succulent and spindly, the fungus survived somewhat longer throughout resistant stems, but no symptoms developed after the initial shock. Histological examination of inoculated plants showed the fungus to be confined to primary xylem of resistant plants while secondary xylem developing around the infected bundle remained free of the fungus. In susceptible plants the fungus spread, in most cases, into secondary xylem. On the basis of these results the authors concluded that the resistant character is one that is well distributed throughout the plant.

[1] Accepted for publication January 16, 1955.

Published with approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the Herman Frasch Foundation and from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

Further insight into the nature of resistance was attained when ethanol (1.0, 0.5, and 0.2 per cent) was added to mineral nutrient solution in which resistant cuttings were rooted after inoculation with the bud-cell suspension. These concentrations of ethanol had no effect on noninoculated cuttings except to retard root formation. The inoculated resistant plants so treated, however, showed severe vascular browning and permanent wilt in 12–15 days.

The present paper presents results obtained in a further extension of the studies of Scheffer and Walker (11).

EXPERIMENTAL RESULTS.—The first experiment was designed to compare the effects of extracts of susceptible Bonny Best and single-gene resistant Jefferson plants on the growth of *Fusarium oxysporum* f. *lycopersici* in synthetic media. Stems were removed from plants of the same age and height and passed through a juice extractor. The juice was collected and centrifuged at low speed to remove the solid particles. Aliquots of the supernatant of both susceptible and resistant varieties were added to 50 ml of a double-strength Czapek-Dox medium in Roux bottles and the volume adjusted to 100 ml with distilled water. The bottles were sterilized by autoclaving, seeded with 1 ml of a spore suspension, and incubated at 28°C for 10 days. At the end of the growth period the mycelial mat was filtered, washed thoroughly with water, dried at 105°C and weighed. Both extracts stimulated the growth of the fungus, but there was no apparent difference between the 2 extracts in this respect. Similar results were obtained in experiments in which the plant extracts were sterilized by filtration.

In plants in which disease symptoms are produced by toxins, resistance might be achieved if the plants possess a mechanism of detoxification. In Fusarium wilt of tomato it has been shown that culture filtrates 2 weeks old are able to reproduce the disease symptoms in susceptible tomato plants. Further, a commercial pectinase preparation did likewise (6). If resistant plants do possess such a mechanism of detoxification, then susceptible and resistant plants treated with

culture filtrates or with commercial pectic enzyme preparations should behave differently. When resistant and susceptible plants were treated with culture filtrates at different dilutions and with a pectic enzyme preparation at different concentrations, both Bonny Best and Jefferson plants reacted similarly. This would indicate that the resistant variety possesses no mechanism to cope with the disease-inducing fungus metabolite. Thus resistance in a resistant plant cannot be explained on a basis of inactivation of the disease-producing metabolite.

The relation of resistance to host metabolism already has been discussed by Scheffer and Walker (11). Certain compounds capable of altering the host metabolism also produce marked variations in the resistance of the host species to Fusarium wilt. Thus maleic hydrazide, which markedly inhibits respiration in higher plants, increases the extent of disease development in susceptible tomato (14). The results of Scheffer and Walker (11) with ethanol are of interest because ethanol is a normal product of anaerobic fermentation in the tomato stem (9). The breakdown of resistance reported could be due to altered permeability of the tissues or to the action of ethanol as a narcotic. Narcotic drugs such as urethanes and higher alcohols, e.g., octyl alcohol, inhibit cellular respiration but they do so only at relatively high concentrations. These compounds also are known to affect the dehydrogenase systems by preventing the reduction of oxidized cytochrome (2). The effects of urethane and ethanol on inoculated Jefferson cuttings were compared. In this and subsequent experiments the inoculation technic of Scheffer and Walker (11) was used. Urethane has no effect on resistance (Table 1). The effect of ethanol, therefore, may be attributed to its action on the permeability of the host cells rather than on host metabolism. Increase in host cell permeability by chloroform leads to an increase in susceptibility to rust (13).

If resistance is related to metabolism, substances which affect metabolism should alter resistance. The compound 2,4-dinitrophenol (referred to hereafter as DNP) is known to affect metabolism profoundly, by uncoupling phosphorylation from oxidation. At 10^{-5} M, DNP has been shown to inhibit almost completely the phosphorylation process, thereby preventing growth and other energy requiring processes, while the respiration rate, as indicated by O_2 uptake, is unaffected or strongly stimulated. Jefferson cuttings were kept in the inhibitor solution (10^{-5}M) for various intervals after inoculaton and then transferred to Hoagland's nutrient solutions. The inoculated controls showed very little wilt and very little vascular browning (Table 2). Plants treated with DNP for as short a period as 3 days following inoculation developed typical disease symptoms.

Newcomb (10) has reported that DNP stimulates respiration of tobacco callus slices, simultaneously releasing aerobic fermentation. Beevers (3) in a study of the effect of DNP on the tissues of a large number of species of plants observed marked stimulation in each of them. The effect of this substance on oxygen uptake by tomato stem slices was studied. The data on respiration reported in Table 3 are based upon conventional Warburg technique and equipment. Stems of Jefferson tomato plants about 6 weeks old were sliced into sections 0.5 mm thick with a hand microtome. The slices were worked by gentle agitation, surface dried on filter paper, and weighed rapidly in a Roller-Smith torsion balance. Usually 500 mg of tissue was used per flask. Oxygen uptake was measured at 30°C with air as the gas phase. Readings were taken at ½-hour intervals over a period of 4 hours. The experiment was run in M/30 phosphate buffer at pH 5.9 which approximates the pH of vascular sap of the tomato. The results in Table 3 show a significant increase in oxygen uptake in presence of 10^{-5} M DNP, the concentration used on inoculated tomato cuttings (Table 2). This suggests that DNP may be

TABLE 1.—*Symptoms in inoculated and noninoculated resistant tomato cuttings treated with ethanol and urethane*

Inhibitor	Conc.	Wilt index[a] Inoc. cuttings	Wilt index[a] Noninoc. cuttings	Browning index[b] Inoc. cuttings	Browning index[b] Noninoc. cuttings
Urethane	10^{-2}M	1.0	0.0	0.6	0.0
Urethane	10^{-3}M	1.0	0.0	0.6	0.0
Ethanol	0.5 per cent	3.8	1.4	2.0	0.0
Water control		1.0	0.0	0.6	0.0

[a] The severity of wilt was determined on an arbitrary basis of 0 to 4, 0 indicating no wilt and 4 indicating maximum wilt, and is presented as the average for 5 plants scored 15 days after inoculation.

[b] The intensity of vascular browning was graded as follows: 0 = no vascular browning; 1 = slight browning at the base of the stem or in some leaf axils; 2 = browning in the lower third of the stem; 3 = browning in the lower two-thirds of the stem; 4 = browning from the base to the apex of the stem. Each figure is the average for 5 plants scored 15 days after inoculation.

TABLE 2.—*Development of disease in inoculated resistant tomato cuttings treated with 10^{-5} M 2,4-dinitrophenol*

Length of treatment (days)	Wilt index[a]	Browning index[b]
0	0.2	0.5
3	2.3	2.5
4	1.7	2.1
8	1.8	2.4
12	2.3	2.4

[a] The severity of wilt was determined on an arbitrary basis of 0 to 4, 0 indicating no wilt and 4 indicating maximum wilt, and is presented as the average for 5 plants scored 15 days after inoculation.

[b] The intensity of vascular browning was graded as follows: 0 = no vascular browning; 1 = slight browning at the base of the stem or in some leaf axils; 2= browning in the lower third of the stem; 3 = browning in the lower two-thirds of the stem; 4 = browning from the base to the apex of the stem. Each figure is the average for 5 plants scored 15 days after inoculation.

TABLE 3.—*Oxygen uptake of tomato stem slices treated with 2,4-dinitrophenol*

Concentration of DNP	Percentage change in O_2-uptake over control
10^{-5}M	+33
5×10^{-5}M	−48
10^{-4}M	−95

causing an uncoupling of phosphorylation from oxidation in the tomato cuttings. Higher concentrations markedly inhibited oxygen uptake.

Phosphorylation is inhibited by DNP and although oxygen consumption is maintained or even enhanced, the energy generated is not available for the endergonic processes of the plant. If the breakdown of resistance is the result of blocking of synthetic processes, respiratory inhibitors might be expected to have an effect similar to that of DNP. Several such inhibitors commonly used to block certain enzymatic reactions in the glycolysis scheme and the tricarboxylic acid cycle were tested in the same manner as that used with DNP. Sodium fluoride was applied for 8 days, thiourea for 12 days, sodium diethyl dithiocarbamate for 6 days, sodium malonate for 9 days, and sodium fluoroacetate for 9 days. Final readings on wilt and browning were taken at the end of 15 days. The first 3 compounds had pronounced effects on breakdown of resistance (Table 4). Treatment with sodium fluoride, which inhibits enolase in the glycolytic system, resulted in an increase in wilting and browning up to 2×10^{-4} M. Use of thiourea, which inhibits ascorbic acid oxidase and polyphenol oxidase, caused an increase in both indices up to 10^{-3} M. Sodium diethyl dithiocarbamate, which also inhibits ascorbic acid oxidase and polyphenol oxidase, increased both wilting and browning to 10^{-4} M. The ineffectiveness of sodium malonate and sodium fluoroacetate might be due to low rate of cell penetration. It is known that the former will penetrate the cell only as the undissociated acid (9). At pH 5.8–6.0, the range at which the experiments were run, malonate would be present in a dissociated form and thus would not be likely to penetrate host cells readily.

TABLE 4.—*Symptoms in resistant tomato cuttings treated with sodium fluoride, thiourea and sodium diethyl dithiocarbamate*

Conc.	Wilt index[a] Inoc. cuttings	Wilt index[a] Noninoc. cuttings	Browning index[b] Inoc. cuttings	Browning index[b] Noninoc. cuttings
	Sodium fluoride			
0	1.0	0.0	0.6	0.0
10^{-4}	2.4		2.0	0.0
2×10^{-4}	2.6	0.0	2.2	0.0
5×10^{-4}	2.0	0.3	1.8	0.0
10^{-3}	2.2	0.3	1.4	0.0
	Thiourea			
0	1.0	0.0	0.6	0.0
2×10^{-4}	1.4	0.0	2.2	0.0
5×10^{-4}	2.0	0.0	2.2	0.0
10^{-3}	2.4	0.0	2.4	0.0
	Sodium diethyl dithiocarbamate			
0	0.0	0.0	0.7	0.2
10^{-3}	1.0	0.0	2.0	0.2
10^{-4}	1.5	0.5	2.2	0.0

[a] The severity of wilt was determined on an arbitrary basis of 0 to 4, 0 indicating no wilt and 4 indicating maximum wilt, and is presented as the average for 5 plants scored 15 days after inoculation.

[b] The intensity of vascular browning was graded as follows: 0 = no vascular browning; 1 = slight browning at the base of the stem or in some leaf axils; 2 = browning in the lower third of the stem; 3 = browning in the lower two-thirds of the stem; 4 = browning from the base to the apex of the stem. Each figure is the average for 5 plants scored 15 days after inoculation.

It was still not determined whether the inhibitors brought about breakdown of resistance through their influence on host metabolism or through a stimulatory effect upon the pathogen. The inhibitors were added in various amounts to Czapek-Dox medium (pH 6.2) in Roux bottles in triplicate and the fungus allowed to grow at 28°C for 10 days. The mycelium was filtered off, dried, and weighed. The results in Table 5 show that all inhibitors tested reduced the amount of growth of the fungus *in vitro*. Breakdown of resistance, therefore, cannot be attributed to stimulation of growth of the pathogen.

Augmented respiration is almost a universal consequence of infection of higher plants with obligate parasites (1). Much of the increased respiration is contributed by the host cells and probably involves oxidation of carbohydrate during the period of vegetative growth of the parasite, since the respiratory quotient is close to 1.0 until the rate of respiration and the growth of the parasite begin to decline. This augmented respiration is believed to be due to the action of an uncoupling agent like DNP. However, since many indications show that the synthetic processes are

TABLE 5. *Growth of the tomato wilt fungus in vitro in the presence of respiratory inhibitors*

Inhibitor	Conc. (molar)	Wt. of mycelium (mg)	Percentage inhibition over control
None		338.0	0.0
Sodium fluoride	10^{-4}	200.0	40.0
	2×10^{-4}	193.0	42.9
	5×10^{-4}	163.0	51.8
2,4-dinitrophenol	10^{-5}	188.0	44.5
	2×10^{-5}	190.0	43.8
	5×10^{-5}	162.0	52.0
Sodium diethyl dithiocarbamate	10^{-4}	215.9	36.4
	5×10^{-4}	180.8	46.5
	10^{-3}	159.4	52.9
Thiourea	10^{-4}	86.0	74.6
	5×10^{-4}	75.2	77.7
	10^{-3}	69.5	79.4

also accelerated in plants infected with obligate parasites, an uncoupling action is not the primary or exclusive cause of augmented respiration. On the other hand, evidence points to the increase in utilization of energy-rich phosphate as the cause of increased respiration (1). The respiration of infected and noninfected Bonny Best stems and infected and noninfected Jefferson stems has been compared over a period of 2 weeks following inoculation. No significant change in oxygen consumption has been observed in either the susceptible or the resistant stems as a result of infection. *Fusarium oxysporum* f. *lycopersici* differs from the obligate parasite in that it does not greatly affect the respiration of the host it infects.

Discussion.—Various attempts have been made by previous workers to determine the fundamental nature of resistance to Fusarium wilt of tomato. Fisher (4), Gottlieb (7), and Irving et al (8) have offered evidence that the expressed sap of resistant plants is inhibitory to the growth of the fungus *in vitro* when compared with the sap of susceptible plants. The results of Snyder et al (12) and those of this investigation have failed to confirm those claims. Variation of concentration and balance of nutrient solution supplied to the host in sand culture were shown by Foster and Walker (5) to have no effect on the expression of single-gene resistance while with multiple-gene resistance the effects were of the same order as those upon susceptible plants.

In this investigation, emphasis has been placed upon the most stable resistance so far known which is that controlled by a single gene and which is represented in the variety Jefferson. Scheffer and Walker (11) showed that the resistant character is well distributed throughout the plant and functions effectively in spite of the fact that the pathogen may by artificial means be introduced throughout the host where it becomes established temporarily in the primary xylem. Winstead and Walker (15) showed that the pectolytic metabolites of the fungus when introduced into the transpiration stream bring about the disease equally well in resistant and in susceptible plants. This leads to the assumption that either the fungus, though established by artificial means in the resistant host, is unable to produce those metabolites effectively, or the host in some way is able to resist the normal action of the fungus as a pathogen.

The results of this investigation indicate strongly that the resistant character is bound up closely with the metabolism of the resistant host. When substances which are known to inhibit phosphorylation or certain steps in the respiratory cycle are introduced into the transpiration stream, the fungus proceeds effectively in producing symptoms identical with those normally produced by natural infection of the susceptible host. It is suggested therefore that in the resistant plant some substance is continuously being formed that accumulates to a level where it becomes toxic to the fungus and blocks its advance. The formation of this substance seems to require energy from phosphorylation reactions, since resistance is quickly lost when these are blocked by 2,4-dinitrophenol. This substance must also be either very labile or continuously metabolized to nontoxic materials, since extracts of resistant plants are not toxic to the fungus. The effect of other inhibitors which break down resistance also could be explained by their action on energy-producing reactions associated with respiratory processes. While certain of the known enzyme processes are interfered with, it does not necessarily follow that they are the ones intimately associated with and responsible for resistance. If they are not, the interference with their normal activity upsets other enzyme processes which are more directly responsible for resistance. Whatever the basic difference between the resistant and susceptible plants may be, it is controlled by a single gene and thus when finally elucidated it may well appear as a relatively simple difference in 1 or a few enzymes in the 2 types of plant.

Department of Plant Pathology
University of Wisconsin
Madison, Wisconsin

LITERATURE CITED

1. Allen, P. J. 1954. Physiological aspects of fungus diseases of plants. Ann. Rev. Pl. Physiol. **5**: 225-248.
2. Baldwin, E. 1952. Dynamic aspects of biochemistry. Cambridge University Press. 544 p.
3. Beevers, H. 1953. 2,4-dinitrophenol and plant respiration. Amer. Jour. Bot. **40**: 91-96.
4. Fisher, P. L. 1935. Physiological studies on the pathogenicity of Fusarium lycopersici Sacc. for the tomato plant. Md. Agr. Exp. Sta. Bul. 374: 261-281.
5. Foster, R. E., and J. C. Walker. 1947. Predisposition of tomato to Fusarium wilt. Jour. Agr. Res. **74**: 165-185.
6. Gothoskar, S. S., R. P. Scheffer, J. C. Walker, and M. A. Stahmann. 1953. The role of pectic enzymes in Fusarium wilt of tomato. Phytopathology **43**: 535-536.
7. Gottlieb, D. 1943. Expressed sap of tomato plants in relation to wilt resistance. (Abs.) Phytopathology **33**: 1111.
8. Irving, G. W., Jr., T. D. Fontaine, and S. P. Doolittle. 1945. Lycopersicin, a fungistatic agent from the tomato plant. Science (n.s.) **102**: 9-11.
9. Link, G. K. K., R. M. Klein, and E. S. Guzman Barron. 1952. Metabolism of slices of the tomato stem. Jour. Exp. Bot. **3**: 216-236.
10. Newcomb, E. H. 1950. Tobacco callus respiration and its response to 2,4-dinitrophenol. Amer. Jour. Bot. **37**: 264-271.
11. Scheffer, R. P., and J. C. Walker. 1954. Distribution and nature of Fusarium resistance in the tomato plant. Phytopathology **44**: 94-101.
12. Snyder, W. C., K. F. Baker, and H. N. Hansen. 1946.

Interpretation of resistance to Fusarium wilt on tomato. Science (n.s.) **103**: 707-708.
13. THATCHER, F. S. 1942. Further studies of osmotic and permeability relations in parasitism. Canad. Jour. Res. Sec. C **20**: 283-311.
14. WAGGONER, P. E., AND A. E. DIMOND. 1952. Effect of stunting agents Fusarium lycopersici and maleic hydrazide, upon phosphorus distribution in tomato. (Abs.) Phytopathology **42**: 22.
15. WINSTEAD, N. N., AND J. C. WALKER. 1954. Production of vascular browning by metabolites from several pathogens. Phytopathology **44**: 153-158.

STRAWBERRY VEIN BANDING VIRUS[1]

Norman W. Frazier[2]

SUMMARY

Strawberry vein banding virus was isolated from a Marshall and 2 hybrid strawberry plants. The variety Alpine proved to be the most useful indicator plant of 30 on which symptoms of the virus was compared.

The virus was transmitted by dodder (*Cuscuta subinclusa*), by grafting, and by aphids. The aphids *Amphorophora rubi*, *Capitophorus fragaefolii*, *Macrosiphum pelargonii*, and *Myzus ornatus* transmitted the virus, but *Aphis gossypii*, *A. rumicis*, *Macrosiphum solanifolii*, and *Myzus solani* failed to transmit it.

Six colonies of strawberry aphids (*C. fragaefolii*), each of which originated from a different locality, were tested for their ability to transmit the virus. Two colonies of dark aphids and 2 colonies of common pale aphids transmitted the virus with efficiency, but 2 other pale aphid colonies failed to transmit. The dark aphids, which were collected on wild woodland strawberry, differed from the pale ones, which originated on cultivated strawberry, in that the abdominal dorsum of the adult apterae were brown.

The incubation period of the disease was usually about 3 weeks.

The virus was acquired during a 30-minute acquisition feeding period but not in periods of 20 minutes. The number of aphids that acquired virus was small for 30- and 60-minute feeding periods and increased sharply up to 2-hour feeding periods.

The virus was transmitted to the first test plant within a 30-minute test feeding period following acquisition feeding. The virus was retained for at least 8 but not 24 hours by aphids which had completed 1-day or 4-day acquisition feeding periods. Transmission was lower after a 4-day acquisition feeding period than following a 1-day period.

Varying the frequency of serial test feeding periods did not affect the maximum period of virus retention. The percentage of transmission increased with increased duration of the test feeding period.

Although vein banding virus is considered to be distinct from any previously described virus, it has certain similarities to viruses 4 and 5 of Prentice and strawberry crinkle virus of Zeller and Vaughan.

During the spring of 1949, a virus was isolated from 2 hybrid strawberry plants growing in a seedling trial bed of the Strawberry Institute of California near Morgan Hill. Vein banding symptoms produced on indicator plants of *Fragaria vesca L.* appeared to be quite distinct. The same year, similar symptoms were found on an indicator plant to which a Marshall variety strawberry plant had been grafted. The Marshall plant had symptoms suggestive of the crinkle disease described in Oregon by Zeller and Vaughan (9). This, and other Marshall plants which subsequently were infected experimentally with the virus by means of aphids, were considered by Harold E. Thomas to be typical cases of the "mild crinkle" which was mentioned by Zeller (8) without a description of symptoms.

The present report summarizes greenhouse studies with this vein banding virus in an effort to characterize and identify it.

METHODS.—Plants of a number of wild strawberry species and varieties propagated either vegetatively in clonal lines or from seed were used as indicators. Seedling plants were transplanted from seed flats to 3-in. pots during the 2-3 leaf stage and were used in tests 7 days or more after transplanting. Daughter plants of clonal lines were established in 4-in. pots and were used as soon after severing from the mother plant as was practicable.

Aphids were colonized and maintained on caged strawberry plants. Noninfective aphid colonies were developed by transferring newly-born aphids, before they had fed, to virus-free plants.

Aphids were transferred from one plant to another by means of a moistened camel's-hair brush. During short test feeding periods, plants were enclosed in glass vials or small jars, whereas for feeding periods of 24 hours or more, cloth-covered wood-framed cages

[1] Accepted for publication February 6, 1955.
[2] The author is indebted to E. O. Essig for identifying the aphids.

were used. At the end of the test period, the plants were fumigated or sprayed and then held for daily observation.

SYMPTOMS.—In order to determine the most suitable indicator plant for vein banding virus, 30 wild strawberry species, varieties, and hybridized seedling selections were compared.

Symptoms were clearest in a runnerless strain of a red-fruited Alpine variety of *F. vesca* (1) which previously had proved to be a useful indicator (2, 4). The author's stock of the variety originated from seed of plants in a private garden. The plants are similar or identical to plants that were grown from commercial seed packaged under the names "Baron von Solemaker" and "Runnerless Harzland." No differences in virus reaction have been detected among the 3 sources. Because Alpine grew well from seed, was suitable for handling in 3-in. pots, and was relatively resistant to fungus infection, it was used as the test host in subsequent experiments.

Alpine.—Vein banding virus causes a relatively mild disease after the plant recovers from initial acute symptoms. Vigor is moderately reduced. Leaf area and petiole length may be reduced by approximately 30 per cent. The first symptoms usually appear on the youngest developing leaf and consists of epinasty of midribs and petiole, a tendency of opposite halves of leaflets to be appressed, irregularly wavy leaflet margins, and slight rugosity or crinkling of lamina. Usually the above symptoms are mild and are not all present simultaneously. As the affected leaf expands, first clearing, then yellowish banding of veins becomes visible. Commonly the yellowing occurs along the main and secondary veins in scattered, discontinuous streaks of varying lengths (Fig. 1, A). Often all veins, including net veins, may be cleared or yellow banded. The second and third leaves formed after onset of symptoms usually are affected more severely than the first or any subsequent leaf. On later-formed leaves the number of chlorotic streaks may be greatly reduced, scattered, and confined to a portion of a single leaflet. Usually plants subsequently will produce a series of completely symptomless, normal-appearing leaves and then symptoms may again appear in either mild or severe form. Affected leaves tend to recover a normal appearance on maturity and the yellow vein banding tends to disappear with leaf age.

Fragaria vesca.—The indicator plant most widely used in strawberry virus research is the East Malling clone of *F. vesca* (5). Symptoms of vein banding virus are clear on this indicator and are similar in all respects to those described on Alpine, differing primarily in that the vein banding is more diffuse or less sharply defined (Fig. 1, C (left)).

In *F. vesca* plants already carrying the strawberry latent virus, strain A (3), symptoms are markedly more severe although of the same type (Fig. 1, C (right)). During the shock stage of the double infection, the petioles are considerably shortened and epinastic; leaflets are recurved toward the petiole; and leaves show severe, usually diffuse, vein yellowing that often results in a general chlorosis of the entire leaf. Although chronic symptoms are much less severe, downward curling and dwarfing of leaflets and shortening of petioles usually persist. Old leaves tend to become reddened or yellowed and prematurely necrotic.

Fragaria californica C. & S. (6).—This host, previously referred to as *F. bracteata* Heller by this author, is quite variable in the wild. It has been used in California and elsewhere as an indicator plant. The sources of the clones used for that purpose have been from plants growing wild either in Sonoma, the source of the test clones used in the present study, or in Santa Cruz County in California. Symptoms produced by vein banding virus on *F. californica* are not well defined, and diagnosis is uncertain. *F. californica* is a nearly symptomless carrier of the virus.

On *F. californica* already carrying strain B of strawberry latent virus, vein banding virus may cause moderate loss of vigor, and mild epinasty of leaflets accompanied by the tendency for the opposite halves of the leaflets to be appressed along the midribs. Infrequently, diffusely chlorotic areas along main veins and faint net vein clearing may occur. Symptoms are not striking even during the shock stage of the double infection.

Marshall.—The Marshall test clone has been demonstrated to be carrying a very mild strain of strawberry latent virus. The symptoms developed on plants experimentally infected by vein banding virus, therefore, were those of a virus complex. They were similar, but somewhat intermediate, to those on Alpine and *F. vesca* carrying strawberry latent virus and inoculated with vein banding virus. The vein banding is usually diffuse, commonly located along main veins, and may often appear as spots. As affected leaves mature, vein banded areas may gradually disappear, or they may become brownish-red or necrotic. Veins frequently become discolored without previous chlorosis, especially on plants out-of-doors. Affected leaflets characteristically exhibit epinasty, mild crinkling, and wavy margins (Fig. 1, B).

TRANSMISSION OF VIRUS.—A single attempt to transmit the virus mechanically by the carborundum method from leaves and stolons of an infected indicator plant to Alpine strawberry, Zucchini squash, Cocozelle squash, Turkish tobacco, Pinto bean, broad-leaved plantain, and sugar beet was unsuccessful. A similar test with juice from roots of the same plant also was unsuccessful.

Two tests of virus transmission were made with dodder, *Cuscuta subinclusa* Dur. & Hilg., as a bridging agent. In each test, strands of dodder growing on a healthy Alpine plant were trained onto a diseased Alpine plant and then onto a second healthy Alpine plant without severing the continuity of the strands. In 1 test the virus was carried through the dodder and infected the second Alpine test plant.

Vein banding virus was transmitted from 3 Marshall

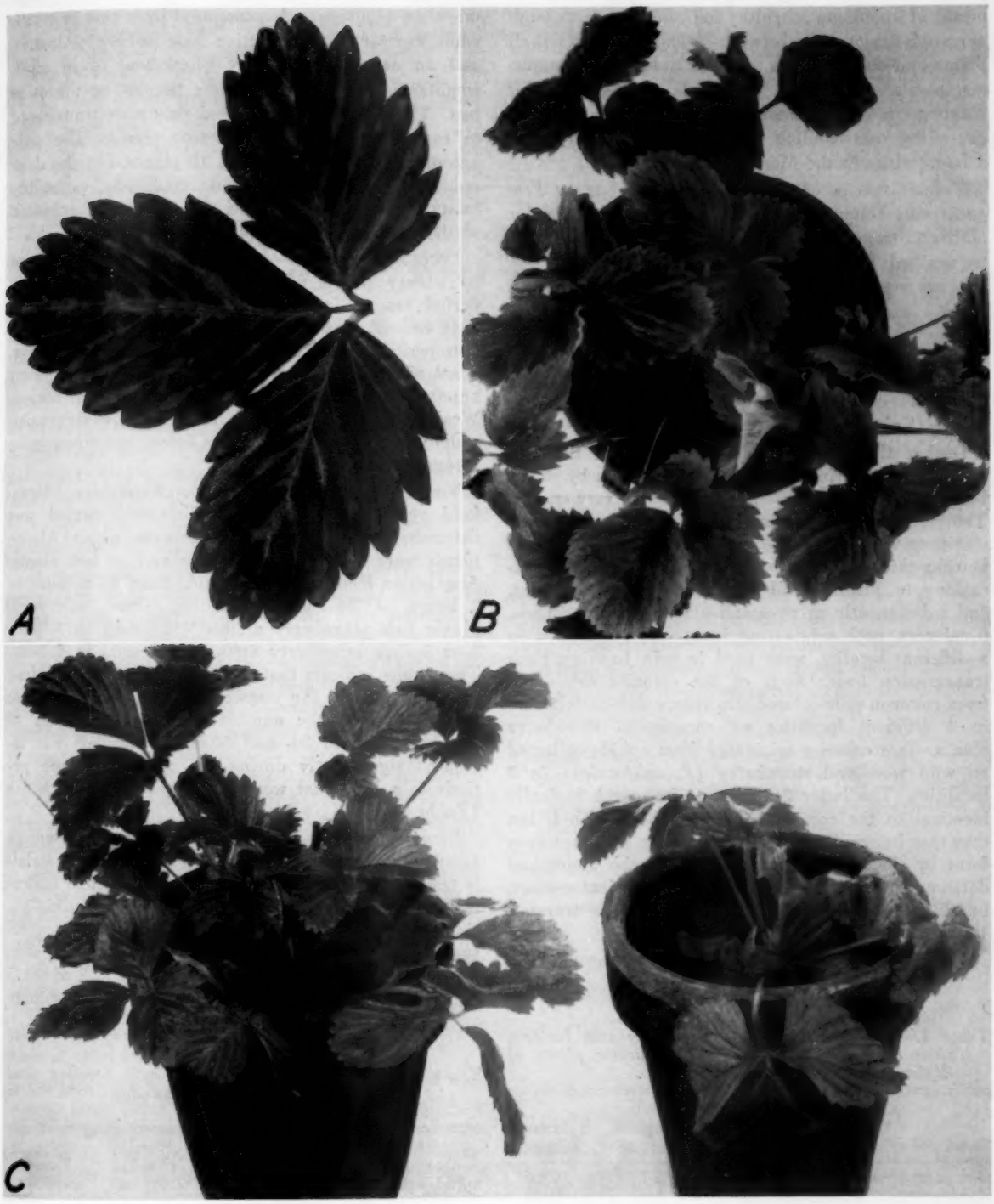

FIG. 1. A) Symptoms of strawberry vein banding virus in leaf of Alpine strawberry showing yellowish banding along the main and secondary veins in discontinuous streaks or spots. B) Marshall strawberry infected with vein banding virus plus strawberry latent virus, showing curled, crinkled leaves with vein chlorosis. C) *Fragaria vesca* (East Malling clone) infected with vein banding virus (left) and with vein banding virus plus strawberry latent virus (right). Note the more severe effect of the virus complex in the plant on the right (horizontal, shortened petioles, severely down-curled and twisted leaflets) in comparison with the plant on the left which shows vein chlorosis and slight crinkling of leaves.

plants, which had been experimentally infected by means of aphids, to 3 healthy indicator plants through approach grafts made between stolons of the Marshall plants and either stolons or floral stalks of the indicator plants. Symptoms produced on the indicator plants were slightly more severe than those typically caused by vein banding virus and were suggestive of a latent virus in the Marshall plants.

Various species of aphids[2] found breeding on *Fragaria* were colonized in the greenhouse and tested for ability to transmit vein banding virus. The virus was transmitted by *Amphorophora rubi* (Kalt.), *Capitophorus fragaefolii* (Ckll.), *Macrosiphum pelargonii* (Kalt.), and *Myzus ornatus* (Laing) (Table 1). Aphid species which failed to transmit the virus, under the conditions of the tests and in a limited number of trials, were *Aphis gossypii* Glover, *Aphis rumicis* Linn., *Macrosiphum solanifolii* (Ashm.), and *Myzus solani* (Kalt.).

During the course of exploratory work that extended over a period of 4 years, transmission by strawberry aphids (*C. fragaefolii*) proved to be very erratic. The virus was sometimes transmitted with high efficiency while at other times all tests were negative. During this period it was necessary on several occasions to abandon all established aphid colonies, and subsequently to re-establish new colonies. Thus, 6 colonies, each originating from aphids collected in a different locality, were used in vein banding virus transmission tests. Four of the colonies originated from common pale-colored strawberry aphids collected in 4 different localities on commercial strawberry plants. Two colonies originated from aphids collected on wild woodland strawberry (*F. californica*) in 2 localities. The latter 2 appear to be morphologically identical to the common pale strawberry aphid, but they may be readily distinguished in the adult apterous form by the dark brown coloring of the abdominal dorsum. In the tests, 4 colonies were efficient vectors, but 2 of the pale aphid colonies failed to transmit (Table 2).

That the failure of 2 of the colonies to transmit vein banding virus was not due to factors associated with inoculum plants was demonstrated by a test in which adult apterae of an inactive pale colony (Albany) and an active dark colony (Berkeley) were given simultaneous acquisition feeding periods on the same leaf. Three aphids of each kind then were transferred to each of 10 separate indicator plants. The pale aphids caused no infection in 10 plants, but the dark aphids infected 9 of 10 plants. Obviously, variability exists among populations of the strawberry aphid in ability to transmit vein banding virus.

INCUBATION PERIOD.—In 154 experimentally infected strawberry plants of various kinds, the incubation period ranged from 14-55 days. The mode was 18 days with 36 periods while 48 per cent of the incubation periods ended between the 16th and 19th days. Plant vigor and degree of severity of first symptoms appeared to affect the length of the incubation period. Weak plants that produced leaves in slow succession usually required longer periods before symptoms were recognized.

VIRUS-VECTOR RELATIONSHIPS.—*Acquisition threshold period.*—The acquisition threshold period was determined in a series of 5 tests in which Alpine plants were used as virus source and as test plants. Acquisition feeding periods varied from 20 minutes to 2 hours. A 24-hour test feeding period was used. Single pale strawberry aphids were used in 1 test; 3 or 5 dark strawberry aphids were used in 4 tests. The results indicate that, although vein banding virus was acquired by the strawberry aphid in 30-minute feeding periods, the number of aphids that did so was low for both 30- and 60-minute periods but increased significantly during 90- and 120-minute periods to a level that was maintained up to 24 hours (Table 3).

Virus retention.—In 2 tests, aphids were given an acquisition feeding period of 24 hours and then serially transferred to 7 successive groups of Alpine indicator plants upon which they were allowed feeding periods of 20, 20, 20, 60, 120, 240, and 960 minutes, respectively. In 1 test in which single pale aphids were tested at each interval on each of 5 plants, 2 of the 5 plants in the 60-minute group and 2 in the 120-minute

TABLE 1.—*Transmission tests of strawberry vein banding virus by 8 species of aphids to indicator plants of Alpine and Fragaria californica*

Species of aphid	Number of virus sources	Number of aphids per test	Transmissions[a]
Amphorophora rubi	5	5-10	9/20
Aphis gossypii	3	5-10	0/23
Aphis rumicis	2	5-10	0/10
Capitophorus fragaefolii	21	4-10	54/110
Macrosiphum pelargonii	6	15	7/30
Macrosiphum solanifolii	1	6	0/3
Myzus ornatus	4	10-20	4/29
Myzus solani	1	10	0/5

[a] The numerator is the number of plants infected; the denominator is the number of plants fed upon by aphids.

TABLE 2.—*Transmission of strawberry vein banding virus by 6 colonies of* Capitophorus fragaefolii *collected in different localities*

Source of aphid colony	Color form	Number of virus sources	Number of aphids per test	Transmissions[a]
El Cerrito	Pale	2	10	0/19
Albany	Pale	8	5-10	0/35
Morgan Hill	Pale	3	5	11/12
Watsonville	Pale	2	5	15/15
Berkeley	Dark	6	4-10	29/31
Stinson Beach	Dark	1	5	7/8

[a] The numerator is the number of plants infected; the denominator is the number of plants fed upon by aphids.

TABLE 3.—*Acquisition of strawberry vein banding virus by Capitophorous fragaefolii during feeding periods of different lengths*[a]

Test	Number of aphids per plant	Length of acquisition feeding periods in minutes[b]								
		20	30	40	60	90	120	240	480	1440
1	1	0/5		0/5	0/5		0/5	0/5	3/5	1/5
2	3	0/2		0/2	0/2		2/2	2/2	2/2	2/2
3	3		0/4		0/4	1/4	4/4	4/4		
4	5		0/10		0/10	3/10	5/10			
5	3		2/30		1/30	6/30	13/30			
Totals		0/7	2/44	0/7	1/51	10/44	24/51	6/11	5/7	3/7

[a] Pale strawberry aphids were used in test 1; dark strawberry aphids were used in tests 2, 3, 4, and 5. Alpine plants were used for virus source and as test plants. A 24-hour test feeding period was used.
[b] The numerator is the number of plants infected; the denominator is the number of plants fed upon by aphids.

group became infected. In the second test in which 3 dark aphids were tested on each of 2 plants during each interval, 1 infection resulted in the 120-minute group. These results indicated a period of retention of at least 2 hours and, in addition, suggested the possibility that either a period of time of about 1 hour following completion of the acquisition feeding period must necessarily elapse before transmission of the virus can occur or that a 20-minute test feeding period is insufficient.

In a second experiment, dark strawberry aphids were given acquisition feeding periods of 1 or 4 days on a single inoculum plant. For each acquisition feeding period, 16 lots of 3 aphids were transferred serially to successive Alpine test plants, with 4 of the lots being used to test each of 4 different test feeding sequences as follows: 1) 8 successive ½-hour test feeding periods; 2) 4 successive 1-hour periods; 3) 2 successive 2-hour periods; and, 4) 1 4-hour feeding period. Then the aphids in all 4 sequences were given an additional 4-hour and finally a 16-hour test feeding period, the latter ending 24 hours after removal of the aphids from the inoculum plant (Table 4).

The virus was transmitted during the first 30-minute test feeding period and was retained longer than 8 hours by aphids of both the 1- and 4-day acquisition feeding period groups. After the 1-day acquisition feeding period, 32 of 92 plants inoculated were infected compared to 19 of 92 plants after the 4-day period. Rather than raising the transmission efficiency of the aphids, the longer acquisition feeding period thus appeared to have had the reverse effect.

In Table 4 the data from the separate tests with aphids given 1-day and those allowed 4-day acquisition feeding periods are combined. In this experiment, varying the frequency of the serial test feeding intervals had no apparent effect on the period of virus retention. Virus retention of more than 8 hours resulted in both the ½-hour and 4-hour test feeding sequences. It is evident that the virus can be transmitted during test feeding periods of 30 minutes. However, the efficiency of transmission increased with increasing duration of the test feeding interval. It is interesting to note that in the 4- to 8-hour test feeding interval (line 9, table 4), during which all aphids were given a 4-hour period on the test plants, virus concentration appears to have been progressively greater in the aphids which had been fed on the fewest test plants during the previous 4 hours.

It is evident that the delay of 1 hour following the acquisition feeding period before transmission that occurred in the preliminary tests did not represent a necessary period. It is probable that transmission during 20-minute feeding periods such as were used during the first hour in those tests could be expected to be infrequent.

In a further test of retention of the vein banding virus in the dark strawberry aphid, aphids were given a 24-hour acquisition feeding period on infected Alpine plants and then transferred serially in 30 lots of

TABLE 4.—*Retention of the strawberry vein banding virus in the dark form of Capitophorus fragaefolii after a 1- or 4-day acquisition feeding period*[a] *and the effect of varying the test feeding period on transmission of the virus to Alpine indicator plants*

Elapsed time from acquisition feeding Hours	Intervals at which transfers of 3 aphids per plant were made during the first 4 hours following acquisition feeding			
	½ hour	1 hour	2 hours	4 hours
½	2/8[b]			
1	0/8	2/8		
1½	1/8			
2	4/8	4/8	4/8	
2½	2/8			
3	0/8	4/8		
3½	0/8			
4	1/8	4/8	6/8	7/8
8	0/8	1/8	2/8[c]	4/8
24	1/8	0/8	0/8	1/8
Totals	11/80	15/48	12/32	12/24

[a] Four of the plants used at each interval received aphids that had completed an acquisition feeding period of one day and the other 4 received aphids with an acquisition period of 4 days. Two lots of aphids in both the ½- and 1-hour transfer series failed to infect any plant.
[b] The numerator is the number of plants infected; the denominator is the number of plants fed upon by aphids.
[c] One aphid died.

3 aphids each to 10 successive Alpine plants. Each lot was allowed to feed 1 hour on each of the first 8 plants, 2 hours on the ninth, and 14 hours on the tenth plant. The ratios of the number of plants infected to the number of plants fed upon by aphids for the 10 successive groups of plants were 9/30, 10/30, 10/30, 7/30, 2/30, 0/30, 0/30, 0/30, and 0/30, respectively. Of 90 aphids used in this experiment, 10 died or were lost, but only 1 each from 3 different lots were lost prior to the ninth transfer. As in the previous test where 1-hour test feeding intervals were used, virus was retained for over 5 hours but not over 6 hours. In both tests there appeared to be a similar flat peak of maximum transmission efficiency extending over approximately the second and third hours which was slightly higher than during the first hour.

RELATION OF VEIN BANDING VIRUS TO OTHER STRAWBERRY VIRUSES.—Vein banding virus appears to be distinct from any described strawberry virus although there are some similarities to viruses 4 and 5 described by Prentice (7), and to crinkle described by Zeller and Vaughan (9).

The symptoms of virus 4 (vein chlorosis virus) on Royal Sovereign strawberry possibly may be very much like those of vein banding virus on Alpine, *F. vesca*, and Marshall. Unlike the vein banding virus, however, virus 4 apparently does not cause definitive symptoms on *F. vesca*. In addition, Prentice was unable to obtain transmission of virus 4 with strawberry aphids. These differences must serve at present to distinguish between virus 4 and the vein banding virus.

Vein banding virus appears to have greater similarity to virus 5 (strawberry leaf-curl virus) than to virus 4. The symptoms described for virus 5 in *F. vesca* are very much more severe than those produced in virus-free *F. vesca* by vein banding virus. However, when vein banding virus is inoculated into *F. vesca* plants already infected by strawberry latent virus, the symptoms of the resulting virus complex very closely duplicate those described for virus 5. The limited amount of comparative data on vector-virus relationships does not serve to distinguish the 2 viruses: the acquisition threshold periods are similar, approximately 1 hour for both viruses; the period of virus retention is of shorter duration for virus 5, about 1 hour in comparison to 8 hours for vein banding virus; and the 5-week incubation period of virus 5 is about twice as long as that of vein banding virus.

Since the Marshall plants that were experimentally infected by vein banding virus were already carrying a mild form of strawberry latent virus, the resulting symptoms, which are equated with Zeller's "mild crinkle," were those of a virus complex. When virus was transmitted from the Marshalls by grafting to indicator plants the symptoms expressed by the indicators were similar to those of strawberry leaf-curl. It appears likely that Zeller's "mild crinkle" and Prentice's strawberry leaf-curl may be related.

The 2 symptoms described by Zeller and Vaughan as characteristic of strawberry crinkle were the crinkled condition and the chlorotic character of the leaves, the latter consisting of pin-point chlorotic spots and of yellow streaks along veins. The symptoms expressed by the experimentally infected Marshall plants agreed with Zeller and Vaughan's account of crinkle in the crinkling and yellow streaks along veins, and also in the occurrence of periods during which symptoms were masked. However, chlorotic spotting was absent except for occasional spots located on veins. Marshall plants that exhibited presumably typical symptoms of strawberry crinkle have been studied and found to differ from the vein banding infected plants in symptomatology and also to differ to some degree among themselves.

It is probable either that Zeller and Vaughan described a complex of diseases or that the name strawberry crinkle has been extended subsequently in usage to apply to more than the originally described disease. Perhaps both suppositions apply. In any event, it appears that vein banding virus is one of perhaps several viruses that undoubtedly have been associated with strawberry crinkle.

DEPARTMENT OF ENTOMOLOGY AND PARASITOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BAILEY, L. H. 1949. Manual of cultivated plants. Rev. ed. Macmillan, New York. 1116 p.
2. FRAZIER, N. W. 1951. Fragaria bracteata Heller as an indicator plant of strawberry viruses. U. S. Dept. Agr. Pl. Dis. Rptr. **35**:127-128.
3. FRAZIER, N. W. 1953. A latent virus of Fragaria vesca. U. S. Dept. Agr. Pl. Dis. Rptr. **37**:606-608.
4. FRAZIER, N. W., AND H. E. THOMAS. 1953. Strawberry a host of western aster yellows virus. U. S. Dept. Agr. Pl. Dis. Rptr. **37**:272-275.
5. HARRIS, R. V., AND MARY E. KING. 1942. Studies in strawberry virus diseases. V. The use of Fragaria vesca L. as an indicator of yellow-edge and crinkle. Jour. Pomol. Hort. Sci. **19**:227-242.
6. JEPSON, W. L. 1925. A manual of the flowering plants of California. Associated Students Store, University of California, Berkeley. 1238 p.
7. PRENTICE, I. W. 1952. Resolution of strawberry virus complexes. V. Experiments with viruses 4 and 5. Ann. Appl. Biol. **39**:487-494.
8. ZELLER, S. M. 1933. Crinkle disease of strawberry. Ore. Agr. Exp. Sta. Bul. 319, 14 p.
9. ZELLER, S. M., AND E. K. VAUGHAN. 1932. Crinkle disease of strawberry. Phytopathology **22**:709-713.

IMMUNITY TO VIRUS X IN POTATO: STUDIES OF CLONAL LINES[1]

Roland G. Timian,[2] W. J. Hooker,[3] and C. E. Peterson[4]

SUMMARY

Susceptible clonal lines of potato that were free of virus X were inoculated with 7 isolates of virus X. The severity of symptoms was then compared. Isolate X5 was consistently effective in inciting both local and systemic symptoms in susceptible clonal lines free of virus X. Almost all susceptible plants developed systemic necrosis or mottle with this isolate. Another isolate, XRS, incited local lesions consistently but was somewhat less effective in inciting systemic symptoms. Less virulent isolates were relatively ineffective for identifying susceptible clonal lines of potato. Isolate X8, which produced no symptoms on plants of *Datura stramonium*, *Nicotiana glutinosa*, *N. tabacum*, and *N. rustica*, was least effective in inciting symptoms in clonal lines of potato.

All isolates of virus X used in the study produced typical local lesions on plants of *Gomphrena globosa*, responded positively in precipitin tests with XRS antiserum, and incited top necrosis in the potato variety Epicure following graft inoculation. Isolate X8 protected plants of *D. stramonium* against more severe isolates of virus X. These isolates, obtained from different sources, varied in their ability to incite symptoms. Certain isolates incited either extremely mild or no discernible symptoms in certain solanaceous hosts other than potato. Others incited mild and others severe reactions in these hosts. In general, severity of symptoms in the potato roughly paralleled that in other solanaceous hosts used for comparison.

Resistance in 169 clonal lines of potato was determined both by mechanical inoculation and by the graft method. The mechanical inoculation test was more rapid and more reliable than the graft test as not all plants within certain clonal lines reacted similarly in a given graft test.

Immune plants in the mechanical inoculation test developed no symptoms following inoculation with any virus X isolate used. These symptomless plants were free of virus X when indexed on plants of *G. globosa*. Susceptible plants usually developed local necrosis or inoculated leaves and often developed systemic necrosis. When plants were inoculated with those isolates that proved most useful in the test, specifically, those producing severe symptoms, systemic necrosis was obtained more often than was systemic mottle.

When inoculated mechanically, more potato plants in a number of clonal lines developed symptoms of systemic infection at 24°C than at 16°, while more plants developed local lesions at 16° than at 24°.

Virus X is one of the most important viruses of potato because of its depressing influence on yield, its universal presence in seed stocks of many varieties, and the difficulty of visual identification of infected plants. The literature concerning the influence of virus X on yield, strain variation in virus X, and types of resistance to virus X in potato has recently been reviewed (3). Techniques have been developed that enable experienced workers to test stocks of susceptible potato for the presence of virus X by serological methods (7), by indexing with suitable plant hosts (4), and by cross-protection tests (1). The incorporation of immunity into potato varieties appears to be the most promising means of controlling virus X.

Considerable attention has been given to the production of potato varieties immune to virus X. This followed the identification of resistance to virus X in unnamed potato seedlings (12) and the demonstration of the heritability of immunity to virus X in seedling S 41956 (13). Schultz, Clark, Stevenson, and Raleigh (11) identified clones immune to virus X by the graft test of Raleigh (9) in which aerial tubers developed on infected scions which had been grafted on immune stocks. This method for identifying immune plants in a potato breeding program limits the number of plants which may be tested because of the time required in grafting and the amount of greenhouse space needed.

This study was undertaken to determine if a rapid and effective method could be developed for identifying plants immune to virus X in a population of potato seedlings. Environmental factors influencing severity of symptoms following mechanical inoculation with a number of isolates of virus X were determined. In order to establish the most suitable method for identifying immune plants (14), a comparison was made between the graft test of Raleigh (9) and a mechanical

[1] Accepted for publication January 20, 1955.
Journal Paper Number J-2602 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1184. This is a joint contribution in cooperation with the United States Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Branch.

[2] Graduate Assistant, Department of Horticulture, Iowa Agricultural Experiment Station. Present address: Department of Agronomy, North Dakota Agricultural Experiment Station, Fargo, N. D.

[3] Associate Professor, Botany and Plant Pathology Department, Iowa Agricultural Experiment Station. Present address: Department of Botany and Plant Pathology, Michigan State College, East Lansing, Mich.

[4] Horticulturist, U. S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Branch. Present address: Horticulture Department, Michigan Agricultural Experiment Station, East Lansing, Mich.

inoculation test in which different isolates of virus X were compared on a number of potato clones free of virus X.

MATERIALS AND METHODS. Stock cultures of virus X isolates were maintained in plants of *Nicotiana glutinosa* L. grown in insect-proof cages in the greenhouse. For inoculum, expressed sap from these virus X infected plants was diluted 1:10 with water to which 400-mesh carborundum (12 g/100 ml) was added.

Potato plants grown from tubers were inoculated when 2–4 leaves were fully expanded. Leaves were rubbed between the thumb and forefinger both of which had been dipped in an inoculum-abrasive mixture. After inoculation, plants were rinsed with a fine spray of water.

Isolates of virus X obtained from several sources were purified by serial passage through the local lesion host *Gomphrena globosa* L. (15). The single-lesion transfer technique as described by Jensen (5) and used by Salaman (10) for virus X was employed. Single lesions were punched from leaves and the tissue was ground with approximately 300 parts of water for each part of leaf material. At least 7 single-lesion serial transfers were made for each isolate. Plants of *G. globosa* were used for single-lesion transfers because greenhouse temperatures at the time the work was done were too high for satisfactory local lesion development in plants of *N. rustica*. After all single-lesion transfers had been made, isolates were transferred first to plants of *N. glutinosa* and later to plants of *N. rustica* at 24°C. On the latter, isolates appeared to be pure in type. Isolate XRS, a severe ring spot strain of virus X made available by Dr. R. H. Larson, Department of Plant Pathology, University of Wisconsin, was used without further purification.

The graft test of Raleigh (9) was made as an approach graft when stems were from 15–20 cm long. Grafting tape was wrapped around the union. Ten days later the Cobbler plant, which had been grown from certified seed, was cut below the graft union and the plant being tested was cut above the graft.

Potato progenies used in this study were from crosses involving a susceptible pollen parent, Cherokee (8), with 3 different immune female parents:

Progeny	Parentage
Iowa 940	B 792-88 × Cherokee
Iowa 941	B 792-94 × Cherokee
Iowa 943	S 41956 × Cherokee

Numbers preceded by B or S are from the U. S. Department of Agriculture Potato Breeding Program.

EXPERIMENTAL RESULTS.—*Origin, symptom expression, and serological tests of several virus X isolates.*— Seven isolates of virus X were obtained from various sources (Table 1). With the exception of isolate X8, mottle symptoms had been expressed in the host from which they were originally taken. After purification by the single-lesion transfer technique, plants of *Gomphrena globosa* L., of *Nicotiana glutinosa* L., of

TABLE 1.—*Virus X isolates, sources, and symptom expression in various hosts*

Isolate number	Original source of inoculum and symptom	Symptom expression in *Nicotiana rustica* 24°C	*Nicotiana glutinosa* 16°C	*Nicotiana glutinosa* 24°C	*Datura stramonium* var. *tatula* 24°C	*Nicotiana tabacum* virus X alone 24°C	*Nicotiana tabacum* viruses X & Y combined 24°C
X4	Triumph, Rhinelander, Wis., severe mottle.	chlorotic spot	none[c]	chlorotic spot and systemic mottle	mottle	mottle	necrotic spot and mottle
X5	B2900-27, Presque Isle, Me., severe mottle.	necrotic ring	necrotic spot followed by systemic mottle	necrotic ring and systemic mottle	mottle and necrosis	necrotic ring	necrosis
X6	Cobbler, Bradley, Minn., strong mottle.	chlorotic spot	chlorotic spot followed by systemic mottle	chlorotic spot and systemic mottle	mottle and necrosis	chlorotic spot	chlorotic spot and necrosis
X7	Red Warba, Northwood, N.D., strong mottle.	chlorotic spot	necrotic spot followed by systemic mottle	chlorotic spot and systemic mottle	mottle and necrosis	chlorotic spot	chlorotic spot and necrosis
X8	Green Mountain[a], Fargo, N. D., no symptoms.	none[c]	none[c]	none[c]	none[c]	none[c]	vein clearing, slight mottle and puckering
X10	Sebago, Rhinelander, Wis., severe mottle.	chlorotic spot	necrotic spot followed by systemic mottle	systemic mottle and chlorosis	mottle and necrosis	chlorotic spot	mottle and veinal necrosis
XRS	[b]	necrotic ring	necrotic spot followed by systemic mottle	clear ring and systemic mottle	mottle and severe necrosis	necrotic ring	veinal necrosis and killing

[a] Isolate X8 was obtained from tubers supplied by Dr. W. G. Hoyman, North Dakota Agr. Exp. Sta., Fargo, N. D.
[b] Isolate supplied by Dr. R. H. Larson. Department of Plant Pathology, University of Wisconsin, Madison, Wis.
[c] Presence of virus X was demonstrated by transfer to plants of *Gomphrena globosa*.

N. rustica L., of *N. tabacum* L., and of *Datura stramonium* var. *tatula* (L.) Torr. were inoculated and observed for symptoms.

On plants of *G. globosa* typical local lesions developed at 24°C within 5 days with all isolates of virus X. With all isolates, the potato variety Epicure exhibited severe top necrosis when grafted with infected plants of *D. stramonium*. These isolates of virus X reacted positively in precipitin tests at dilutions of 1–32 and 1–64 when partially clarified sap was tested with XRS antiserum. Abundant flocculation was obtained within 10 minutes at 50°C when a 1–40 dilution of XRS antiserum (titre 1–512) was mixed with an equal volume of clarified juice from infected plants. Juice from leaves and stems of infected plants of *N. glutinosa* was heated for 15 minutes at 55°C and then centrifuged for 15 minutes at 3000 rpm. There were no apparent differences among these isolates in any of the above tests.

Plants of *D. stramonium* inoculated with isolate X8 were protected in cross protection tests conducted 21 days later from the isolates which incited severe symptoms. A synergistic effect was evident on Havana 38 tobacco with all virus X isolates in combination with potato virus Y, isolate 12, obtained from R. H. Larson (2). In the latter double infections, symptoms were less severe following infection with isolate X8 than with other isolates tested.

Isolate X8 was very mild in its effect on plants, producing no symptoms in a number of solanaceous hosts (Table 1), and appearing to be very similar to the isolate X^H of Salaman (10). On the hosts tested, isolates XRS and X5 incited more severe symptoms than did any of the others. These 2 isolates were the only ones that sometimes killed plants of *D. stramonium*.

Symptom expression in potato clones inoculated with different isolates of virus X.—Isolates X5, XRS, X7, X4, and X8 were compared to determine which isolate was most suitable for evaluating potato progenies segregating for immunity to virus X. From 3 different progenies (Ia 940, Ia 941, and Ia 943) 37 clones, 6 immune and 31 susceptible, were selected for testing. These clones were representative of a larger group of 169 clones that had been tested previously by mechanical inoculation with isolate XRS and by the graft test. They had been grown in isolation from other potatoes to maintain the susceptible stocks free from virus X. From each clone, groups of 3 plants were inoculated with each of the 5 isolates and grown at 16°C. Similar groups of inoculated plants were grown at 24°.

Symptoms on each plant were recorded at weekly intervals for 7 weeks (Fig. 1). Local lesions consisted of necrotic localized spotting in inoculated leaves. Systemic symptoms incited by virulent cultures such as X5 and XRS usually were necrotic lesions on the noninoculated leaves similar to those shown by Ladeburg et al (6). This often was followed by severe top necrosis. Only occasionally were mild mottle symptoms obtained.

Of particular significance were the marked differences among isolates in production of local and systemic symptoms. Almost all susceptible plants inoculated with isolate X5 had local and systemic symptoms within 2 weeks at 24°C, but systemic symptoms were somewhat retarded at 16°. With isolate X5, all susceptible plants except 2 exhibited systemic symptoms within 4 weeks at 16°. Isolate XRS was very effective in the identification of susceptible clones by local lesion symptoms at either temperature. In marked contrast to isolate X5, XRS was relatively ineffective in inciting systemic symptoms. Only 41 per cent of the plants exhibited systemic infection at 24° during the 7-week period of observation, and at 16° still fewer plants showed systemic infection. Isolate X7 was somewhat inferior to X5 and XRS in inciting local lesions. It was less effective than X5, but it was considerably more effective than XRS in inciting systemic infection.

With all isolates, local lesions were incited either earlier or in a higher percentage of plants at 16°C than at 24°. This difference was greater with the isolates that incited mild symptoms, X7, X4, and X8, than with X5 and XRS. Since only 60 and 75 per cent of the susceptible plants inoculated with X8 and X4, respectively, had local symptoms, these isolates were not considered suitable for use in identifying susceptible clones. For the development of systemic symptoms, 24° was generally more favorable than 16°. The number of plants with systemic symptoms following inoculation with isolates X8 and X4 was again so low that no real differences between temperatures were evident.

Comparison of 2 methods for identifying immune potato plants.—Up to the present, the graft test of Raleigh (9) has been used as a standard means of identifying plants immune to virus X. In the present study, this method was compared with one involving mechanical inoculation of potato plants free of virus X followed by observation of such plants for symptoms.

Five seed pieces from each of 169 clones of potato from progenies Ia 940, Ia 941, and Ia 943 were planted in 3-in. pots and 2 plants of each were inoculated mechanically with XRS. An inoculated plant with a noninoculated control was placed in each of 2 greenhouses, 1 at 15°C and 1 at 20°. Plants were reinoculated 1 week later.

One noninoculated plant of each clone was approach-grafted to Cobbler plants infected with virus X and held at 15°C. These plants later were moved outside where the temperature fluctuated from 15° to 25°. Symptoms were recorded at weekly intervals over a 7-week period.

Mechanically inoculated plants were observed at weekly intervals during the same period. Some susceptible individuals had necrotic symptoms in inoculated leaves only, whereas others developed systemic

necrosis and mottle. In some instances the reaction was so severe that plants were killed. Plants with either local or systemic symptoms were considered susceptible whereas plants showing no symptoms were considered probably immune. Tubers from symptomless plants were harvested at normal maturity and tested for virus X on plants of *G. globosa* by the method of Hoyman (4). Attempts to recover virus X from these symptomless plants following the mechanical inoculation test were uniformly negative, suggesting that the plants were immune.

The formation of aerial tubers in scions above nonnecrotic stocks, considered to be an immune reaction (11), was found reliable in these tests (Table 2). Plants responding in this way failed to develop symptoms when inoculated mechanically with suitable isolates of virus X. Thus, the 2 methods of identifying immune clones were in agreement. Furthermore, the susceptible plants responding by necrosis of the stock with no formation of aerial tubers in the graft test, consistently developed symptoms of infection in the mechanical inoculation test. The 2 methods of testing for immunity were again in good agreement.

Incomplete agreement between the 2 methods was obtained with plants that produced no aerial tubers above nonnecrotic stocks and with those that produced aerial tubers above necrotic stocks. The majority of the 63 clones in these groups were susceptible to virus X. Of these, 8 gave a susceptible reaction in the graft test and an immune reaction following mechanical

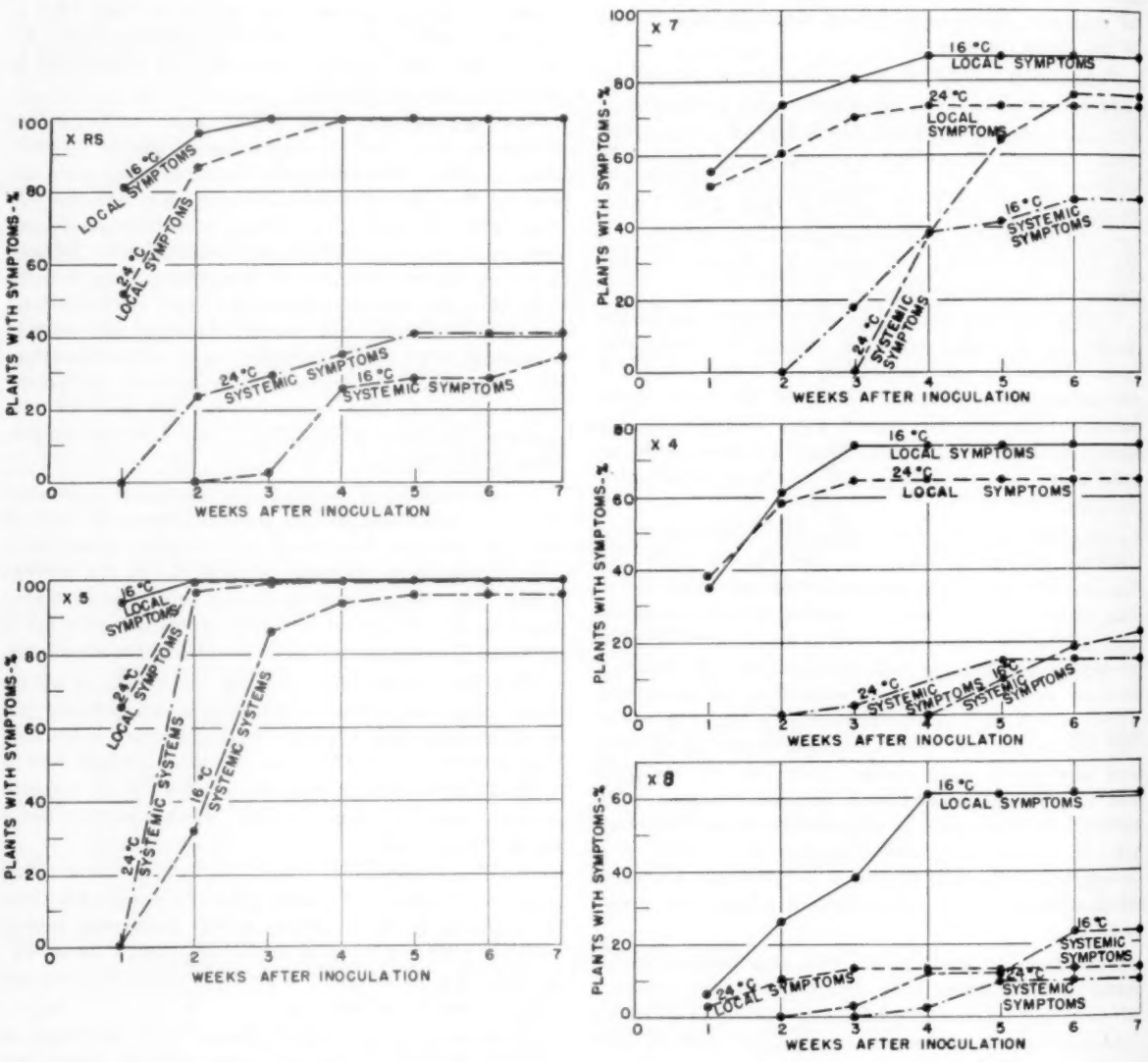

FIG. 1. Frequency of symptom expression with 5 isolates of virus X in 31 susceptible clonal lines of potato inoculated and incubated at 2 temperatures.

TABLE 2. *Distribution of symptoms in 169 clonal lines of potato tested for immunity to virus X by mechanical inoculation and by grafting*

Symptoms in the graft test	Progeny number	Symptoms following mechanical inoculation: local lesions only	systemic symptoms	no symptoms	Total clonal lines tested
		no.	no.	no.	no.
Aerial tubers, nonnecrotic stock	Ia 940	0	0	24[a]	24
	Ia 941	0	0	34[b]	34
	Ia 943	0	0	19	19
	total	0	0	77	77
No aerial tubers, necrotic stock	Ia 940	4	8	0	12
	Ia 941	4	11	0	15
	Ia 943	0	5	0	5
	total	8	24	0	32
No aerial tubers, nonnecrotic stock	Ia 940	1	11	0	12
	Ia 941	1	19	4[c]	24
	Ia 943	0	11	0	11
	total	2	41	4	47
Aerial tubers, necrotic stock	Ia 940	1	0	0	1
	Ia 941	2	3	1[c]	6
	Ia 943	2	4	0	6
	total	5	7	1	13

[a] Includes 2 clones that were reclassified after a second test. Results in the first test were similar to those indicated except that with these 2 clones no aerial tubers were formed in the graft test.
[b] Includes 1 clone that was reclassified after a second test. Results in the first test were similar to those indicated except that the stock was necrotic in the graft test.
[c] Insufficient seed of these 5 clones to permit a second test.

inoculation. It is probable that these plants were immune since virus X could not be recovered from them after the second inoculation. Furthermore, virus X was not recovered from leaves of these clonal lines after plants had been grafted to Cobbler plants that were infected with virus X. Since seed supply of 5 of the aberrant reactors was exhausted, further testing was not possible. However, in each of 3 clonal lines tested a second time, a few individuals developed aerial tubers typical of immune plants.

Raleigh (9) observed that, following good aerial tuber formation, the underground parts of the stocks of immune plants grafted with Green Mountain scions carrying virus X developed poorly and became necrotic early. Raleigh made no mention of above-ground necrosis without aerial tubers similar to that present in 32 of the clonal lines here tested (Table 2). It seems very probable that above-ground necrosis might logically follow necrosis of subterranean portions of the plant.

In order to determine the uniformity of the reaction in more detail, Epicure, Red Warba free of virus X, 25 unnamed susceptible clones free of virus X (Fig. 1), and 5 unnamed immune clones were selected for further testing with both methods. Mechanical inoculation tests with X5 and XRS were made on these clonal lines at 16° C and at 24°. After the graft union was established and the stems cut, half of the grafted plants were held at 16°C and half at 24° for 7 weeks. Since there were no appreciable differences between the graft reactions at the 2 temperatures nor in the mechanical inoculation tests at the 2 temperatures, the combined data are presented (Table 3).

In this second test (Table 3), 18 of the 19 immune plants (group 1) responded in a typical manner by aerial tuber formation above a nonnecrotic stock. Only 1 plant gave a susceptible reaction by failure to develop aerial tubers above the graft union.

Virus X was demonstrated in the leaves of the 125 susceptible individuals from the 27 clonal lines in the graft-inoculation test (Table 3, groups 2–9). In the graft test, only 8 susceptible clonal lines (group 2) consistently responded as expected with neither necrosis nor aerial tubers. The tolerant variety Red Warba was in this group. A hypersensitive reaction in which the stock became necrotic was consistent in 9 susceptible clonal lines (group 3), which included Epicure. Epicure is a variety from Great Britain resistant to virus X by virtue of its hypersensitivity. The necrotic condition of the stocks of plants in group 3 suggests a degree of sensitivity to virus X somewhat greater than that observed in clonal lines of group 2 (Fig. 2).

Considerable variability was encountered in the

TABLE 3.—*Reaction of immune and susceptible clonal lines to 2 different methods of testing for resistance to virus X*

Kind and number of group	Clonal lines tested	Reaction in mechanical inoculation test[a]		Reaction in graft test				
		plants tested	plants infected	plants tested	no. reaction	necrosis of stock	necrosis of stock with aerial tubers	aerial tubers only
no.	no.	no.	no.	no.	no.	no.	no.	no.
immune								
1	5	57	0	19	1	0	0	18
susceptible								
2	8	92	92	35	35	0	0	0
3	9	104	102[b]	40	0	40	0	0
4	2	19	19	8	6	2	0	0
5	1	12	12	5	2	2	1	0
6	3	36	36	17	0	12	5	0
7	2	23	21[b]	11	0	8	1	2
8	1	11	11	5	1	2	1	1
9	1	12	12	4	1	0	0	3

[a] Combined results of inoculation with virulent isolates X5 and XRS.
[b] A few individuals escaped infection with XRS at 24°C. There were no escapes at 24° with X5 or at 16° with either isolate.

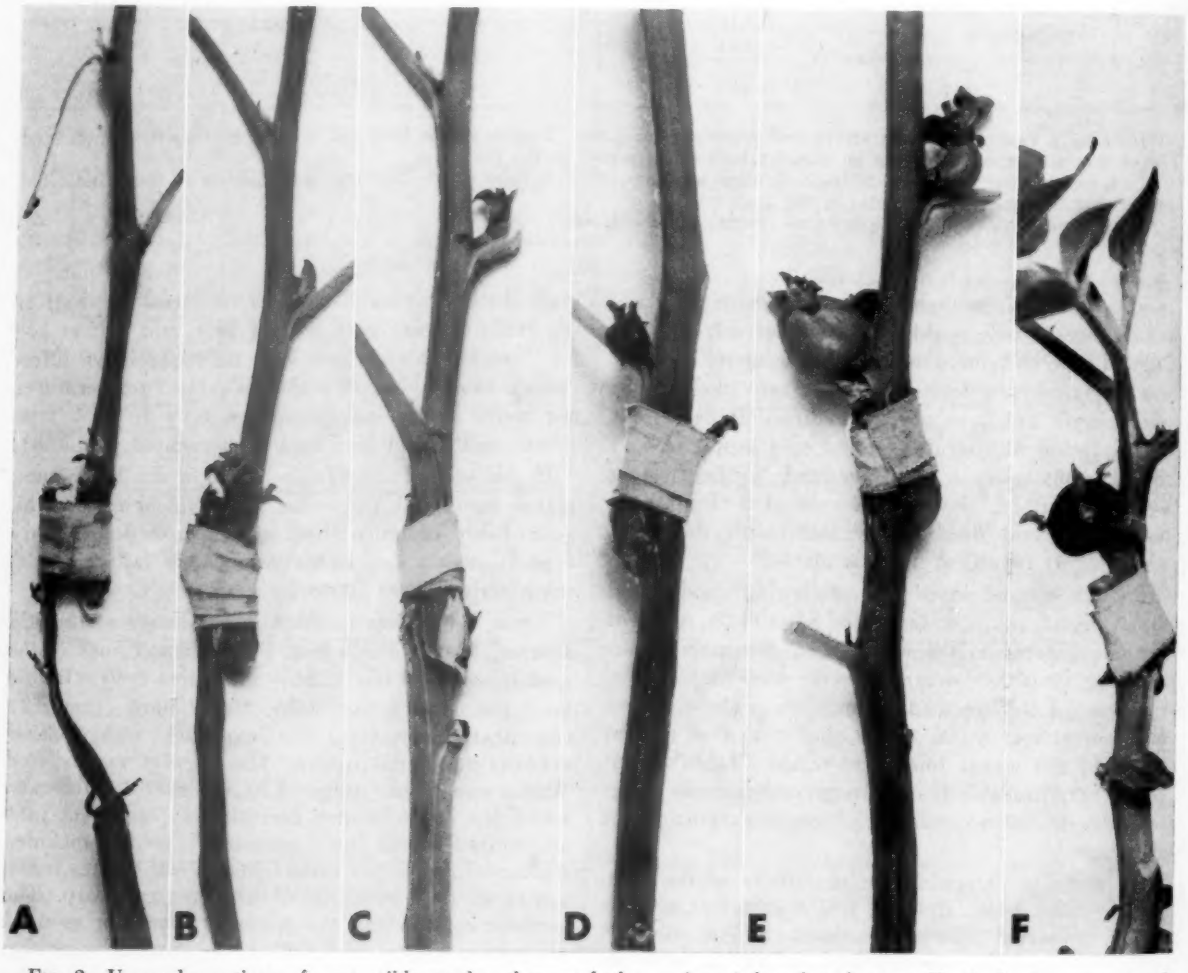

FIG. 2. Unusual reactions of susceptible stocks when grafted to scions infected with virus X: A, B, C) necrosis of stock without aerial tuber formation in the scion; D, E) aerial tuber formation above necrotic stock; and F) aerial tuber formation above a nonnecrotic stock. The reaction of F is generally typical of immune rather than of susceptible stocks. Photographs by R. E. Wicklund.

graft test with the 10 susceptible clonal lines in groups 4–9. In these groups, individual plants within a clonal line reacted differently from the others in the line. Of the 50 plants tested by grafting, 10 reacted as typically susceptible with neither necrosis of the stock nor aerial tubers, 26 developed necrotic stocks, and 8 developed aerial tubers above necrotic stocks. It is noteworthy that 6 susceptible plants developed aerial tubers above stocks that were not visibly necrotic. This reaction is typical of immune types rather than of susceptible types.

Thus, susceptible individuals within a clonal line responded quite differently in the graft test when used as stocks with Cobbler scions that were infected with virus X. It should be pointed out that although the Cobbler scions were grown from certified seed, no precautions had been taken to establish uniform strains of virus X within the seed lot. The probability is great that the number of strains or combinations of strains of virus X differed widely in the several Cobbler plants. Since differences among strains influenced to a considerable degree the number of susceptible plants with symptoms following mechanical inoculation (Fig. 1), it is probable that the strain or strains of virus X present in the Cobbler scions likewise influenced the degree of necrosis in susceptible stocks.

In a few instances a small portion of the Cobbler stock was left below the graft union at the time approach grafts were cut. Aerial tubers developed at the basal portion of such cut stems regardless of whether the stock was immune or susceptible.

IOWA STATE COLLEGE
AMES, IOWA

LITERATURE CITED

1. BAWDEN, F. C., B. KASSANIS, AND FLORENCE M. ROBERTS. 1948. Studies on the importance and control of potato virus X. Ann. Appl. Biol. **35**: 250-265.
2. DARBY, J. F., R. H. LARSON, AND J. C. WALKER. 1951. Variation in virulence and properties of potato virus Y strains. Wis. Agr. Exp. Sta. Res. Bul. 177, 32 p.
3. HOOKER, W. J., C. E. PETERSON, AND R. G. TIMIAN. 1954. Virus X resistance in potato. Amer. Potato Jour. **31**: 199-212.
4. HOYMAN, W. G. 1951. A method of indexing potatoes for virus X by use of petiole or tuber juice. Amer. Potato Jour. **28**: 713-721.
5. JENSEN, J. H. 1933. Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic. Phytopathology **23**: 964-974.
6. LADEBURG, R. C., R. H. LARSON, AND J. C. WALKER. 1950. Origin, interrelation and properties of ringspot strains of virus X in American potato varieties. Wis. Agr. Exp. Sta. Res. Bul. 165, 47 p.
7. MUNRO, J. 1954. Maintenance of virus X-free potatoes. Amer. Potato Jour. **31**: 73-82.
8. PETERSON, C. E., N. K. ELLIS, R. V. AKELEY, AND F. J. STEVENSON. 1954. Cherokee: a new medium-maturing potato variety resistant to common scab, late blight, mild mosaic and net necrosis. Amer. Potato Jour. **31**: 53-58.
9. RALEIGH, W. P. 1936. An abnormal graft reaction in potato resulting from virus infection of a scion on a resistant stock. Phytopathology **26**: 795-796.
10. SALAMAN, R. N. 1938. The potato virus 'X': its strains and reactions. Phil. Trans. Royal Soc. London. Ser. B. **229**: 137-217.
11. SCHULTZ, E. S., C. F. CLARK, F. J. STEVENSON, AND W. P. RALEIGH. 1937. Resistance of the potato to latent mosaic. Amer. Potato Jour. **14**: 124-127.
12. SCHULTZ, E. S., AND W. P. RALEIGH. 1933. Resistance of potato to latent mosaic. (Abs.) Phytopathology **23**: 32.
13. STEVENSON, F. J., E. S. SCHULTZ, AND C. F. CLARK. 1939. Inheritance of immunity from virus X (latent mosaic) in the potato. Phytopathology **29**: 362-365.
14. TIMIAN, R. G. 1953. Identification of seedling potatoes immune to virus X. (Abs.) Phytopathology **43**: 487.
15. WILKINSON, R. E., AND F. M. BLODGETT. 1948. Gomphrena globosa, a useful plant for qualitative and quantitative work with potato virus X. (Abs.) Phytopathology **38**: 28.

PRUNUS TOMENTOSA AS AN INDEX PLANT FOR SOUR CHERRY VIRUSES [1]

Harry C. Fink [2]

SUMMARY

Acquiring quantitative information about virus in cherry nursery stock requires a reliable index plant that is readily available in large quantities. The data presented indicate that *P. tomentosa* is a reliable index plant for cherry virus, both in the greenhouse and in the field.

In greenhouse comparisons, *P. tomentosa* seedlings and Montmorency cherry trees were inoculated with 19 sources of necrotic ring spot, 3 sources of prune dwarf, and 1 source of peace rosette mosaic. Both index plants reacted to all sources of necrotic ring spot and prune dwarf. *P. tomentosa* reacted to peach rosette mosaic while Montmorency cherry did not.

In the field, indexing on *P. tomentosa* resulted in detection of virus in more of the trees indexed than did indexing on Elberta peach. All of 111 Early Richmond cherry nursery trees were found to be infected by indexing on *P. tomentosa;* only 45 were found with peach. Ten of 119 seed trees of *P. mahaleb* were found to be infected by indexing on *P. tomentosa,* whereas 7 were so found by indexing on Elberta peach.

The 3 distinct types of symptoms induced on *P. tomentosa* by fall field budding with Montmorency cherry and later transfer to the greenhouse suggested the possibility of thus distinguishing viruses, complexes, or strains on *P. tomentosa,* but such possibility was not confirmed.

In these experiments indexing on *P. tomentosa* resulted in detection of necrotic ring spot and other viruses in cherry at least as readily as did indexing on Montmorency cherry, perhaps more so, and much more readily than indexing on Elberta peach. Since *P. tomentosa* is usable as easily-grown 1-year-old seedlings, its usefulness as an index plant for determination of presence or absence of virus in large numbers of cherry trees is clearly indicated.

There has been need for a suitable, readily available plant for indexing large numbers of sour cherry, *Prunus cerasus* L., in the program for producing virus-free tree stocks in southwestern Iowa nurseries. Sour cherry, peach, and flowering cherries (*P. serrulata* Lindl. vars. Shirofugan and Kwanzan) have been used as index plants on the basis of data submitted by Moore (5), Hildebrand (3) and Cation (1), and Milbrath and Zeller (4), respectively. The flowering cherries are not winter-hardy in Iowa and peach is not suitable for greenhouse indexing. Sour cherry is satisfactory from these standpoints but is propagated by budding, which requires 2 years of time after setting *P. mahaleb* L. understock seedlings, which themselves at times contain virus.

Nanking cherry, *P. tomentosa* Thunb., can be propagated from seed, is available from nurseries as seedlings apparently in virus-free condition, and is winter-hardy in Iowa. It exhibits recognizable symptoms, especially in the greenhouse, and usually in the field, apparently as reliably as sour cherry and more so than peach.

REPORTED INDEX PLANTS FOR SOUR CHERRY VIRUS.—For this presentation, the term, "cherry virus" refers to the sour cherry yellows complex, which involves an initial shock symptom commonly referred to as necrotic ring spot. From an index standpoint, the currently accepted basic assumption is that when buds of an "unknown" fail to induce necrotic ring spot symptoms, the "unknown" is free of cherry yellows.

Hildebrand (3) reported peach to be a satisfactory index plant for cherry yellows when budded during the summer and immediately pruned back to induce new shoot growth. Cation (1) has used the criteria, delayed spring growth and die-back and rosetting of peach seedlings, to indicate presence of necrotic ring spot and yellows, respectively, in cherry buds grafted to peach seedlings the previous fall. Milbrath and Zeller (4) have reported detection of cherry virus by grafting buds to Shirofugan and Kwanzan varieties of flowering cherry. Necrosis and gumming at point of bud insertion and malformation of new shoots, respectively, are the indicative symptoms on these 2 plants.

The method of Moore and Keitt (5) is of particular interest because it utilizes the plant of immediate concern, sour cherry, and because it utilizes the phenomenon of latency, which makes indexing necessary. Moore and Keitt demonstrated that when a virus-containing bud is grafted to a virus-free sour cherry, more or less violent necrotic ring spot symptoms occur within about 2 weeks. Furthermore, when a virus-containing bud is grafted to a tree already containing virus, no necrotic ring spot symptoms are induced. Thus it is possible to index a particular tree by both a direct and an indirect method.

The essential equivalence of *P. tomentosa* to virus-

[1] Accepted for publication December 29, 1954.
Journal paper No. J-2574 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1060. Taken from a thesis submitted to the graduate faculty, Iowa State College, in partial fulfillment of requirements for the degree, Doctor of Philosophy.

[2] Assistant Plant Pathologist, North Carolina Agricultural Experiment Station.

free sour cherry and its superiority to peach in expressing necrotic ring spot have been briefly reported (2). The following is a somewhat more detailed report.

COMPARISON OF PRUNUS TOMENTOSA WITH CHERRY FOR GREENHOUSE INDEXING.—In the winter of 1950, 19 lots of sour cherry budwood known to contain virus capable of inciting necrotic ring spot were obtained from Wisconsin cherry orchards through the courtesy and aid of Dr. J. D. Moore. Thirteen, 2, 1, and 3 of these lots were known to be capable of inducing cherry yellows, recurrent necrotic ring spot, green ring yellows, and necrotic ring spot, respectively. In addition, Mr. Donald Cation supplied from Michigan 1 budwood lot with peach rosette mosaic virus and 3 lots with prune dwarf virus. One prune dwarf source was known to induce necrotic ring spot on peach and one to induce "questionable" symptoms on peach.

Each of the 19 Wisconsin sources was budded into 5 potted Montmorency cherry trees and 5 potted *P. tomentosa* seedlings in the greenhouse, 2 buds per tree or seedling, at time of bud break. Similarly each of the 3 Michigan sources of prune dwarf and the 1 Michigan source of peach rosette mosaic were budded into 2 Montmorency cherry and 2 *P. tomentosa*.

Symptoms appeared on new growth, which occurred somewhat more rapidly on *P. tomentosa* than on Montmorency cherry. On *P. tomentosa*, the basic symptoms were chlorosis and necrosis. Chlorotic areas on the leaf lamina were either ring-like or diffused and irregular; they varied in size from small spots to areas that included the entire leaf. 'Oak leaf' patterns and 'vein banding' were in some instances clearly discernible. Necrotic rings; necrotic leaf areas, round or irregular; necrotic entire leaves; and necrosis of growing stem terminals occurred before, during or after chlorosis. The necrotic rings, leaf areas and stem terminals were similar to those on Montmorency cherry, though considerably more pronounced. Symptoms induced by Wisconsin source B-3-22 on *P. tomentosa* and Montmorency cherry are shown in Fig. 1.

Occurrence of these symptoms on the budded *P. tomentosa* and Montmorency cherry was observed and recorded periodically. After 2 months, symptom development was considered complete. Summarized final results are in Table 1.

All *P. tomentosa* seedlings developed symptoms when budded with any of the 23 virus sources. It is evident that from the standpoint of 'detection,' symptom ex-

FIG. 1. Necrotic ring spot on *P. tomentosa*, A and B, and Montmorency cherry, C, induced by Wisconsin source B-3-22, necrotic ring spot and yellows.

TABLE 1.—*Reaction of Prunus tomentosa and Montmorency cherry to 19 sources of necrotic ring spot, 3 sources of prune dwarf and 1 source of peach rosette mosaic. Greenhouse, 1950*

Virus source	Description	Trees budded	Trees with symptoms: Montmorency cherry	Trees with symptoms: *Prunus tomentosa*
G-1-5	NRS + Y	5	5	5
G-1-7	NRS + Y	5	5	5
G-1-13	NRS + Y	5	5	5
G-1-19	NRS + Y	5	5	5
G-2-5	NRS + Y	5	5	5
G-2-7	NRS + Y (M)	5	5	5
G-17-4	NRS + Y	5	5	5
G-20-5	NRS + Y	5	5	5
B-1-4	NRS + Y	5	5	5
B-3-23	NRS + Y	5	4	5
M-3-17	NRS + Y	5	5	5
M-7-74	NRS + Y (M)	5	4	5
B-3-22	NRS + Y (R)	5	5	5
M-6-19	NRS + Y (R)	5	5	5
S-5009	NRS + Y (R)	5	5	5
G-2-1	NRS	5	5	5
G-5-1	NRS	5	4	5
B-1-12	NRS	5	5	5
M-5-74	NRS (G)	5	5	5
Lyall 883	P. D.	2	2	2
Young 875	P. D. (P)	2	1	2
Elkhorn 881	P. D. (Q)	2	1	2
Elkhorn 813	P. R. M.	2	0	2

NRS + Y: Necrotic ring spot and yellows.
(M): Reported to be mild on cherry.
(R): Reported to be recurrent on some hosts.
NRS: Necrotic ring spot.
(G): Green ring mottle.
P. D.: Prune dwarf.
(P): Does not produce ring spot on peach.
(Q): Produces none or questionable symptoms on peach.
P. R. M.: Peach rosette mosaic.

pression was fully as reliable on *P. tomentosa* as on Montmorency cherry for all Wisconsin sources and for Michigan sources of prune dwarf. Furthermore, Elkhorn 813, Michigan source peach rosette mosaic, induced symptoms on *P. tomentosa* but not on Montmorency cherry.

Three of the 5 *P. tomentosa* seedlings budded with each of the 19 Wisconsin sources were observed in detail for types and severity of symptoms induced. Although there was some consistent variation among sources, such observation of symptoms did not enable clear separation into 'groups' (i.e., necrotic ring spot only, necrotic ring spot and yellows, necrotic ring spot and yellows—recurrent, and necrotic ring spot and green ring mottle).

COMPARISON OF P. TOMENTOSA WITH ELBERTA PEACH SEEDLINGS FOR FIELD INDEXING.—On July 7, 1949, buds from each of 111 Early Richmond sour cherry trees were grafted to 3 Elberta peach and 2 *P. tomentosa* seedlings growing in the nursery at Shenandoah. One week after bud insertion, all seedlings were pruned back to 1 or 2 nodes above the inserted bud. In all cases, at least 1 or 2 green leaves were left intact on the seedling cane. There were symptoms on new growth of both peach and *P. tomentosa* approximately 1 month after budding. For checks, every fifteenth seedling was left unbudded and every sixteenth seedling was budded with a known source of necrotic ring spot. Buds from all the 111 Early Richmond sour cherry trees induced typical symptoms on *P. tomentosa;* buds from only 45 did likewise on peach.

Furthermore, of 119 *P. mahaleb* seed trees similarly indexed, 7 were positive according to peach index, whereas the same 7 plus 3 additional were positive on *P. tomentosa.* Indexing on peach and *P. tomentosa* indicated presence of virus in the same 4 of 8 wild black cherry (*Prunus serotina* Ehrh.), none of 20 Waneta plum and none of 20 trees of *Prunus besseyi* Bailey.

Symptoms on *P. tomentosa* in the field were predominantly chlorosis, with or without some necrosis. Chlorosis consisted of chlorotic rings, chlorotic areas

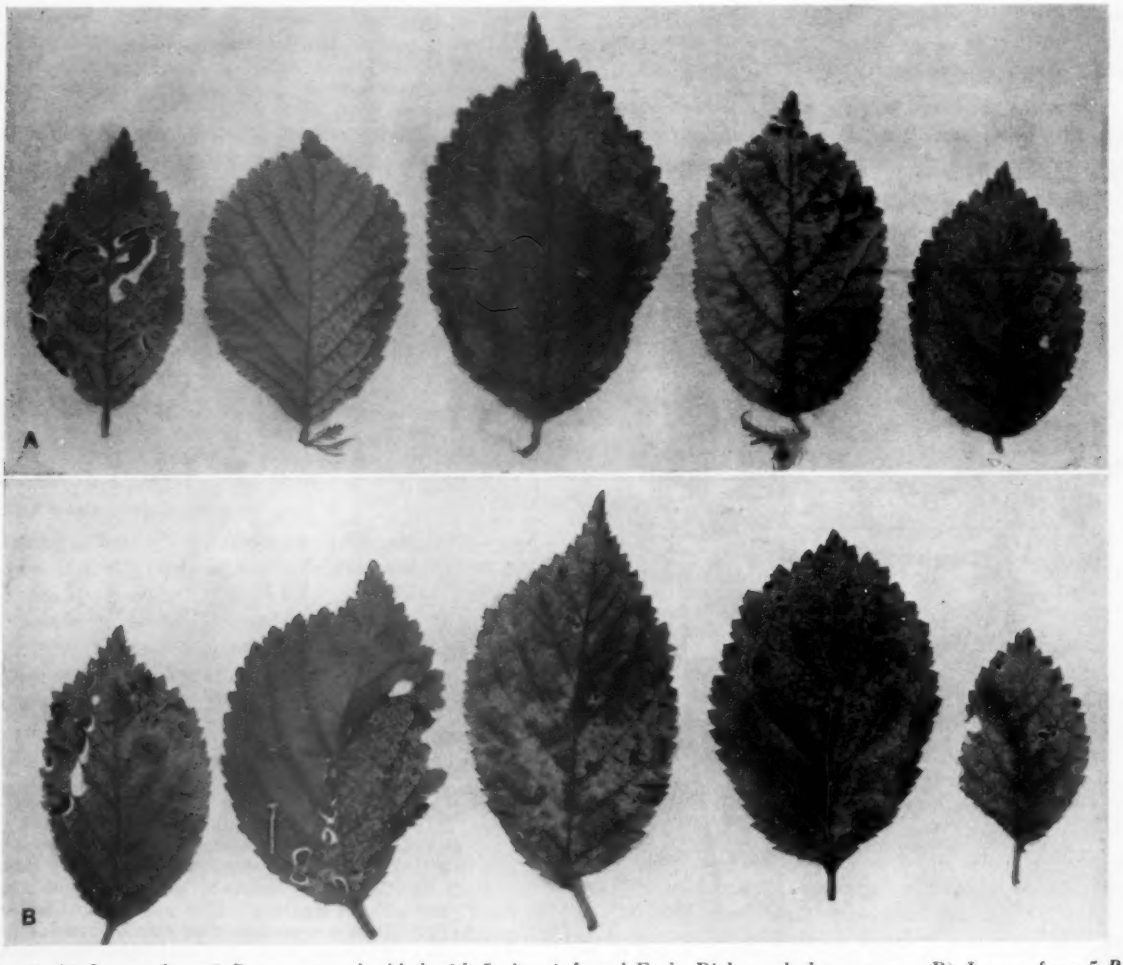

FIG. 2. A) Leaves from 5 *P. tomentosa* budded with 5 virus-infected Early Richmond cherry trees. B) Leaves from 5 *P. tomentosa* budded with 5 virus-infected wild black cherry trees.

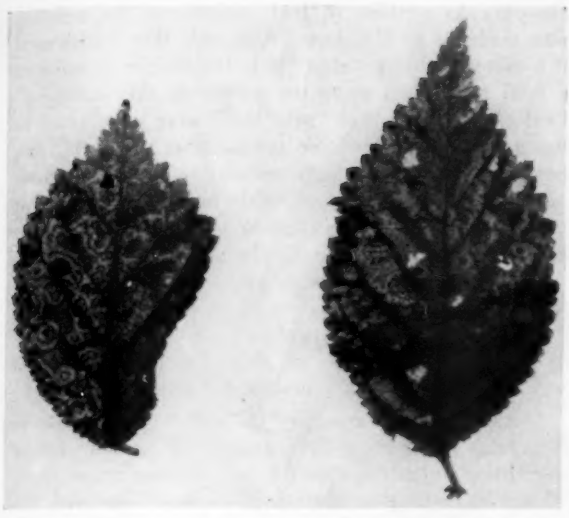

FIG. 3. Symptoms on *P. tomentosa* leaves induced by virus carried in buds from *P. mahaleb* seed trees.

including 'oak leaf' patterns, and 'vein banding.' Necrosis, when present, consisted primarily of necrotic rings, ending in 'shot-hole'. There was only slight stunting and rosetting of new growth, whereas these were the predominant symptoms on peach seedlings, where they were often, though not always, associated with necrotic rings on the leaves. Symptoms induced on *P. tomentosa* by virus in Early Richmond sour cherry, wild black cherry, and *P. mahaleb* orchard trees are shown in Fig. 2 and 3.

VIRUS SYMPTOM EXPRESSION ON P. TOMENTOSA FALL BUDDED IN THE FIELD AND MOVED INTO THE GREENHOUSE. —Some Montmorency cherry buds were inserted in seedlings of *P. tomentosa* in the field on September 1, 1949. These trees failed to grow after being cut back, so were dug and 'heeled in' on October 10. On December 1 they were dug, potted and placed in the greenhouse. Symptoms on new growth were observed and recorded a month later.

Infected buds induced symptoms of 3 distinct and consistent types: 1) necrosis of the budded cane with chlorosis and mottling and necrotic rings on foliage of new canes growing from the roots, 2) rugose distortion and stunting with necrosis along the main veins of new leaves on the budded cane, and 3) chlorosis and mottling and necrotic rings of new leaves on the budded cane. In all cases, duplicate plants of *P. tomentosa* with inserted buds from the same Montmorency cherry tree developed similar symptoms. Wood from trees of *P. tomentosa* with these 3 types of symptoms was grafted on dormant, virus-free Montmorency cherry trees; all induced similar necrotic ring spot symptoms on the newly unfolding cherry leaves. Whether or not these 3 distinct symptom types were ascribable to 3 distinct viruses, complexes, or strains was not further probed.

IOWA STATE COLLEGE
AMES, IOWA

LITERATURE CITED

1. CATION, D. 1942. Cherry yellows in Michigan. Mich. State Hort. Soc. Ann. Rept. **72**: 115-116.
2. FINK, H. C. 1950. Prunus tomentosa as an index plant for necrotic ring spot of sour cherry. (Abs.) Phytopathology **40**: 9.
3. HILDEBRAND, E. M. 1942. Indexing cherry yellows on peach. Phytopathology **32**: 712-719.
4. MILBRATH, J. A., AND S. M. ZELLER. 1945. Latent viruses in stone fruits. Science **101**: 114-115.
5. MOORE, J. D., AND G. W. KEITT. 1949. An indexing method for necrotic ring spot and yellows of sour cherry. (Abs.) Phytopathology **39**: 15-16.

A PEACH DISORDER POSSIBLY RELATED TO THE VIRUS BUD FAILURE DISEASE OF ALMOND[1]

E. E. Wilson and H. Keith Wagnon[2]

SUMMARY

Peach trees in central California are affected by a disorder which is characterized by stiffly erect foliage and failure of buds to grow. A similar disorder developed in young peach trees 3 years after they were inoculated with buds from almond trees affected by Drake almond bud failure, a virus disease.

Since 1944, plant pathologists have reported 3 stonefruit tree disorders that are characterized by failure of buds to grow. So far as it is known, 2 of these, almond bud failure and Drake almond bud failure, occur only on almond; the third, willow twig, occurs only on peach. Drake almond bud failure and willow twig are caused by graft-transmissible viruses, whereas almond bud failure has not been shown to be caused by a graft-transmissible agent despite many attempts to do so (2, 3, 4, 5, 6, 7, 8).

This paper is concerned with a peach disorder with some characteristics of peach willow twig but with most of the symptoms that are shown by almond trees affected by Drake almond bud failure.

[1] Accepted for publication February 6, 1955.
[2] Department of Plant Pathology, University of California, Davis, and Bureau of Plant Pathology, California Department of Agriculture, Sacramento, respectively.

Because of its effect on leaf posture, the new peach disorder is tentatively called "mule's ear." It may be recognized by the stiffly erect and often partially folded leaves that develop on affected branches in early June. This posture is in contrast to the outward and downward curvature of the leaves on normal trees (Fig. 1, A, B). Furthermore, affected leaves are more tapered at the apex than normal ones and usually are somewhat lighter green in color.

In autumn the leaves are retained on the trees for some time after the normal ones have fallen. At this time, therefore, the disorder is most easily recognized in the orchard.

In the spring, many of the buds that were produced in the axils of leaves the previous summer fail to grow (Fig. 2). In other cases, however, no macroscopically visible buds will have formed in the upper portion of the current growth. Such a condition is not to be confused with the bud failure condition which results from insufficient chilling of the buds during the winter period.

Many trees affected by the mule's ear disorder exhibit symptoms only on branches of 1 scaffold limb. Sometimes symptoms are seen on 1 or 2 branches of several scaffold limbs; at other times the tree exhibits symptoms throughout the top. The number of branches with symptoms increases slowly from year to year. In 1 orchard where the twigs with symptoms were removed during fall pruning operations, the trees failed to show leaf symptoms on twigs produced the following summer.

Affected branches produce less fruit than normal ones and the fruits that develop mature a week or so later than is normal for the variety. Consequently, growers regard affected trees as nontypical for the variety. Some call them "wild" or "rooster" trees. On clingstone varieties, an occasional fruit produced on affected branches may be lopsided or otherwise malformed because of uneven development and irregular maturity of various portions of the flesh. With Faye Elberta, the only freestone variety found affected to date, as much as 10 per cent of the fruit remaining on an affected branch may be malformed. In several instances, malformed fruit were found on otherwise symptomless branches of affected trees.

Only one mention of a peach or nectarine disorder with the leaf posture characteristic of mule's ear has been found in the literature. Shamel, Pomeroy, and Harmon (1) described a condition called "Peak wild-leaf strain" or simply "wild leaf" that they found in Merced County, California, on Peak, Sims, Gaume, and Halford No. 2 varieties. Their description and photographs pertain to leaf symptoms; no mention was made of bud failure. Although they considered it a mutation, they stated "It is impossible to come to a final conclusion as to the nature of the variation." Leaf characteristics of "wild leaf" were manifested in the growth from buds or scions from affected trees. Sometimes, however, the branches produced from buds of affected trees "reverted to the normal character of foliage." Progeny trees made a weak growth and several died in the orchard the first year after propagation. No fruit was found on affected branches of such trees, whereas symptomless branches bore normal crops. For the most part, plant pathologists have overlooked the description of this disorder because it was reported to be a mutation.[3]

In the course of a survey[4] of budwood source trees in 1952, Mr. James Breece and the junior author found the mule's ear disorder on the same property on which Shamel, Pomeroy, and Harmon observed the wild leaf disorder 26 years earlier. It was found on trees of the Gaume, Sims, and Peak varieties which had been planted after Shamel and his associates made their studies.

In field surveys during 1952, 1953, and 1954, 50 cases of mule's ear were found on 15 properties in 8 counties in California. Varieties affected included 1 freestone peach (Faye Elberta), 9 clingstone peaches (Carolyn, Corona, Gaume, Halford No. 2, Johnson, Paloro, Peak, Sims, and Stanford), and 1 nectarine (Early Le Grand). The greatest number of affected trees found on any 1 property was 24 in a 5-year-old orchard of Faye Elberta peaches.

The principal features of willow twig are the dying and shedding of lateral fruit and leaf buds. The main terminal bud of the branch usually survives, but it produces a growth that is abnormally slender and flexible. The fruit do not mature properly on these branches and the leaves persist in autumn after those on normal branches have fallen (4). The new disorder resembles the willow twig disease in its effects on the buds and on leaf-fall in autumn. Some affected trees develop the long willowy "hanger" branches similar to those produced on trees affected by willow twig, but not all do so. Moreover, stiffly erect foliage has not been reported as a symptom of willow twig.

[3] The authors are grateful to Mr. George Dobbins, Nursery Service, California Department of Agriculture, for bringing this publication to their attention.

[4] Work done under Agricultural Marketing Project Number RM: C-3550-27, for which state funds were matched with federal funds received from the Agricultural Marketing Service, United States Department of Agriculture, under provisions of the Agricultural Marketing Act of 1946.

→

FIG. 1. The posture of the leaves on: A) A peach twig affected by the mule's ear disorder and B) a twig from a non-affected peach tree. C) The normal posture of almond foliage contrasted with D) the erect foliage produced on almond trees affected by the virus type of bud failure.

A
C
D

FIG. 2. Branch from a seedling peach tree inoculated with the virus type of almond bud failure. Stiffly erect foliage and failure of buds were symptoms that developed 3 years after inoculation.

The disorder known as "Drake almond bud failure" was first reported in 1944 (5), but it was more fully described in 1947. At that time it was reported to be a virus disease, differing from the more common almond bud failure which has not been demonstrated to be caused by an agent that can be transmitted by budding or grafting (2, 7). Drake almond bud failure produces on almonds most of the characteristic symptoms of the mule's ear disease of peach. The stiffly erect foliage, (Fig. 1 C, D), the failure of the buds in the axils of such foliage, the retention of the foliage until late in autumn, and malformation of the fruit are all symptoms of Drake almond bud failure.

The earliest attempts to transmit the virus causing Drake almond bud failure to peach trees were in 1942. Parallel inoculations of almond in that year resulted in symptoms in 1944, but no symptoms developed in the inoculated peach trees. Because the land was needed for other purposes the peach trees were removed in the fall of 1944. Another series of inoculations was made into seedling peach and almond trees in 1949. The inoculum consisted of buds from 3 separate lots of almond trees affected by the Drake almond bud failure type of disorder. Five buds from each source were placed in each of 5 seedling peach trees in August, 1949, and in 5 trees of each of 3 varieties of almonds (Peerless, Nonpareil, and Jordanolo). In the spring of 1951, some trees of each variety of almond had bud failure symptoms and by 1952, 12 of the 15 inoculated trees had symptoms. No recognizable signs of transmission to the peach trees were found until August, 1951, when stiffly erect foliage was noted in certain branches in 1 or 2 trees of each inoculated lot. By 1952, 14 of the 15 peach trees bore 2 or more branches with erect foliage. Subsequently, many buds on such branches failed, and all or most of the branches of certain trees bore erect foliage. No symptoms appeared on the 25 noninoculated peach trees growing in the same row with the inoculated trees.

Symptoms induced in the seedling peach trees resembled, in all major respects, those associated with the mule's ear disorder occurring on several varieties of peach in orchards of central California. Whether or not the seedling peach disorder and mule's ear are identical remains to be proved. Further studies are necessary to clarify the relation between willow twig and the mule's ear disorder.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA
AND
BUREAU OF PLANT PATHOLOGY
CALIFORNIA DEPARTMENT OF AGRICULTURE
SACRAMENTO, CALIFORNIA

LITERATURE CITED

1. SHAMEL, A. D., C. S. POMEROY, AND F. N. HARMON. 1932. Bud variation in peaches. U. S. Dept. Agr. Cir. 212, 21 p.
2. STOUT, G. L., AND E. E. WILSON. 1947. Studies of a bud failure condition in almond trees. (Abs.) Phytopathology **37**:364.
3. THOMAS, H. E. 1949. Willow twig of peach and nectarine. Calif. Fruit and Grape Grower **3**(7):11.
4. THOMAS, H. E. 1951. Willow twig. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Handb. 10:95-97.
5. WILSON, E. E., AND G. L. STOUT. 1944. A bud failure disorder in almond trees. Calif. Dept. Agr. Bul. 33:60-64.
6. WILSON, E. E., AND G. L. STOUT. 1950. Observations on the bud failure disorder in Jordanolo, a new variety of almond. (Abs.) Phytopathology **40**:970.
7. WILSON, E. E., AND G. L. STOUT. 1951. Drake almond bud failure. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Handb. 10:191-194.
8. WILSON, E. E. 1952. Development of bud failure in the Jordanolo variety of almond. (Abs.) Phytopathology **42**:520.

HOSTS, TRANSMISSION, AND ELECTRON MICROSCOPY OF CYMBIDIUM MOSAIC VIRUS WITH SPECIAL REFERENCE TO CATTLEYA LEAF NECROSIS [1]

D. D. Jensen and A. H. Gold

SUMMARY

Cymbidium mosaic virus is shown to cause 3 distinct types of disease in the hosts Cattleya, Cymbidium, and *Datura stramonium* L. In Cattleya and related orchids the disease is called Cattleya leaf necrosis and is characterized by rings, streaks, and other irregular sunken areas of brown to black necrotic tissue in the leaves. The first symptoms usually appear in from 4–6 weeks. Typically the symptoms in Cattleya become conspicuous on the older leaves and are only occasionally evident on the youngest growth. Older leaves may be killed within a few months after infection, and thereafter such plants may fail to show reliable symptoms. Flowers produced on diseased plants appear to be normal in form and color, but premature death of leaves due to virus infection results in fewer and smaller flowers.

In Cymbidium the disease is called Cymbidium mosaic because the mosaic leaf mottle is the most characteristic symptom. Brown necrotic areas also develop in the older leaves of many plants. The virus becomes systemic in both Cymbidium and Cattleya.

Datura stramonium was shown to be susceptible to the virus. In this host the virus does not become systemic, but produces local lesions on the older inoculated leaves in 14 days.

The virus is transmissible by juice inoculation in the presence of carborundum, by rubbing juice on the leaves with the fingers in the absence of an abrasive, by razor blade cuts through drops or a film of infective sap, and by pruning shears.

In electron micrographs, Cymbidium mosaic virus particles derived from necrotic Cattleya, from mosaic Cymbidium, and from experimentally infected *D. stramonium* were similar in form and size.

A virus disease of Cattleya orchids, characterized by necrotic areas in the leaves, has been investigated in California since 1951. In 1953, Jensen (6) indicated that this disease in Cattleya is caused by Cymbidium mosaic virus (4, 5). Surveys and transmission tests show that the virus infects many species and hybrids of Cattleya and such closely related orchids as Laeliocattleya and Brassocattleya, in addition to Cymbidium and Epidendrum.

Necrosis is widespread in Cattleya throughout California in commercial orchid ranges, and is causing considerable concern among growers. Cattleya leaves from plants in Oregon, in Colorado, and in Cuba proved to carry the same virus. In 1952, Murakishi (9) reported a virus disease of Cattleya in Hawaii causing "stunting, distortion, and appearance of reddish-brown to blackish pitted areas and sunken streaks in the leaves and pseudo-bulbs". Dr. Murakishi, when shown some of the infected California plants in June, 1952, stated that the disease was probably the same as that encountered in Hawaii. Orchid growers from widely separated regions of the country state that the same symptoms have been observed on plants in other parts of the United States. It is thus highly probable that the disease in both Cattleya and Cymbidium is cosmopolitan.

The incidence of the disease varies within collections with certain lots of plants having more infection than others. For example, a group of hybrids having *Laeliocattleya lustre plumosa* as one of the parents had a high incidence of infection compared with the rest of the orchids in the range.

Some growers have attributed the symptoms in Cattleya to cold wet weather. This is not an illogical conclusion, because there is some evidence that the development of symptoms may be favored by the environmental conditions obtaining during the winter months.

The advanced necrotic symptoms of the disease in some Cattleya plants are suggestive of a fungus or bacterial disease. However, Dr. P. A. Ark, of the University of California, Berkeley, was unable to isolate pathogenic fungi or bacteria from diseased tissue. Experimental transmission of the virus to Cattleya and to Cymbidium and electron microscopy indicate that the disease is caused by a virus.

This paper reports transmission and electron microscope studies of Cymbidium mosaic virus and its effects on other orchids and on *Datura stramonium* L.

SYMPTOMS. — *Cattleya and related genera.* — The characteristic symptoms on plants in commercial greenhouses are sunken brown-to-black leaf patterns of various forms (Fig. 1, A). In a few plants there are coalescing rings with dead centers surrounded by normal tissue or necrotic rings with green centers (Fig. 1, D). More commonly, the necrotic tissue develops as irregular elongated streaks occurring primarily on the older leaves. The younger shoots occasionally have discernible, but less conspicuous symptoms. The symptoms are typically more evident on the under surface of the leaf than on the upper surface, but as more and more of the internal tissue collapses and dies, the dark discoloration becomes visible through the upper surface (Fig. 1, B; Fig. 2, A). This may occur without the upper surface developing sunken areas.

When the virus kills leaves prematurely, diseased

[1] Accepted for publication January 28, 1955.

plants produce fewer and smaller flowers which are, nevertheless, normal in form and color and which lack the variegation associated with the infection of Cattleya with some viruses. However, the presence of virus in symptomless flowers can be demonstrated by inoculation of healthy test plants with extracted flower juice.

Mature plants of the hybrid *Laeliocattleya shoshone* experimentally infected by juice inoculation developed symptoms in the older leaves in from 4–6 weeks. The first symptoms consisted of faint to conspicuous chlorotic streaks and rings 6–12 mm in diameter (Fig. 1, C). This chlorosis was followed by internal collapse and necrosis of the leaf tissue near the lower epidermis, with resultant sunken spots and streaks. In some cases, the chlorosis remained faint for several weeks and necrosis did not develop until 3–5 months after inoculation. In those plants, the necrotic areas were limited in extent. In other plants, the disease progressed so rapidly that entire older leaves had become chlorotic and necrotic and had dropped within 2–3 months after inoculation. Within 5 months after inoculation, 2–5 of the oldest leaves had been killed. The younger leaves showed only slight symptoms. In another experiment in which very young Cattleya seedlings were inoculated mechanically, the first symptoms developed in 5–6 weeks as water-soaked areas on the leaves. Three weeks later the water-soaked regions of the leaves were collapsed, and brown sunken areas developed on both the lower and upper leaf surfaces. Some of these leaves subsequently dropped prematurely.

Some Cattleya plants develop only mild symptoms and these may be limited to 1 or 2 older leaves, or 1 or more leaves may be severely affected and drop prematurely without symptoms appearing in the other shoots. Since the flowers are symptomless, it is thus possible for Cattleya plants to be infected without showing visible evidence of the disease.

Symptoms on Cymbidium.—Symptoms of Cymbidium mosaic in Cymbidium were described in detail previously (4, 5, 6). Approximately 4–6 weeks after infection, areas of tissue develop a lighter-than-normal green color in the basal visible portion of the younger leaves of a new shoot. As the leaves mature, some may develop extensive areas of reddish-brown tissue in the form of streaks and round patches, which appear first on the under surface of the leaves (Fig. 2, B). The flowers remain symptomless.

Transmission.—The test plants used in the transmission experiments were symptom-free Cattleyas and Cymbidiums. Most of the Cattleyas were young seedlings growing in thumb pots or in pots 2–4 in. in diameter. Some of the larger seedlings were beginning

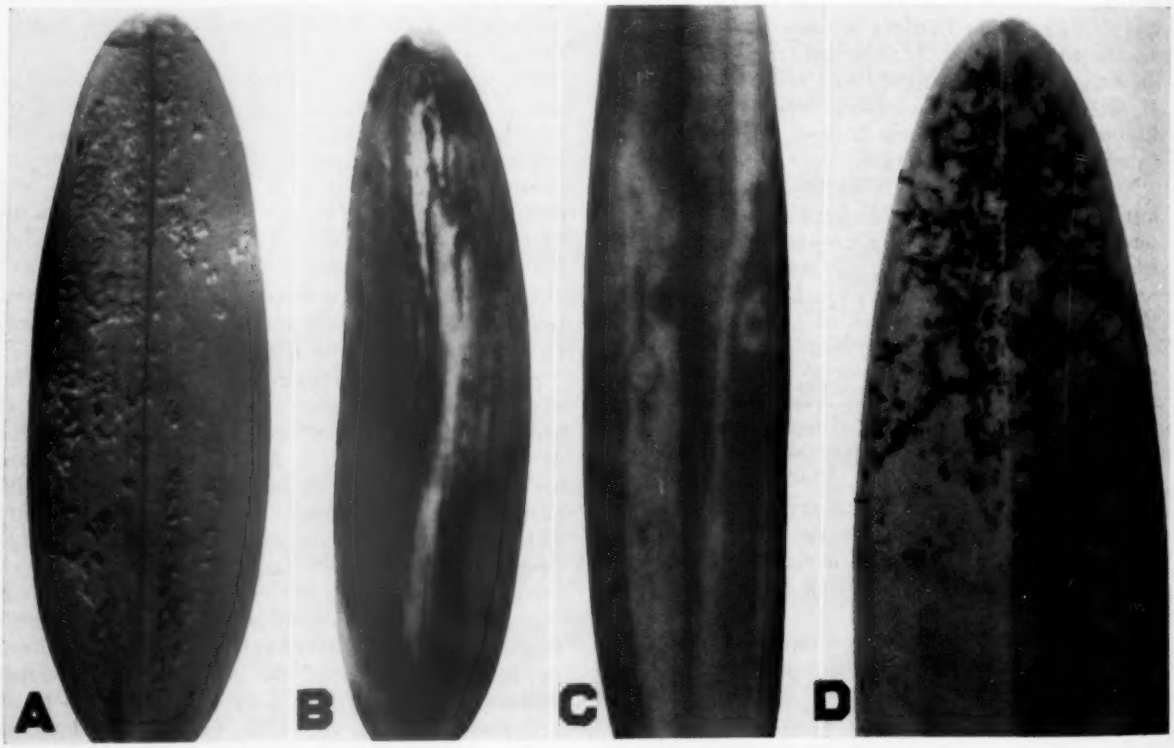

Fig. 1. Symptoms produced by Cymbidium mosaic virus on leaves of Cattleya. A) Light brown pits and irregular areas of sunken tissue on under side of leaf; partial rings in basal portion. B) Extensive internal necrosis (black) and chlorosis (white) in leaf shortly before premature death. C) Chlorotic streaks and rings seen through upper surface of leaf of experimentally-infected *Cattleya shoshone*. D) Necrotic rings on under surface of Cattleya leaf.

to flower. The other Cattleya test plants were older plants large enough for commercial production of flowers. It now appears probable that a few of these older Cattleyas were already infected with virus before use in these trials but were no longer showing symptoms; this could explain failure of symptom development in some of the inoculated test plants listed as negative. Five plants with symptoms were found among several thousand young Cattleya hybrid seedlings examined in commercial houses; also, 2 of 50 noninoculated Cattleya plants developed symptoms within a few weeks after they had been obtained from a grower. These were seedlings under 5 years of age. All of the Cymbidium test plants were young seedlings with only a single shoot, or with 1 bulb and 1 new shoot. No disease developed in a large number of noninoculated Cymbidium control plants.

Cymbidium mosaic virus was found to be transmissible to healthy Cattleya and Cymbidium plants when the leaves of the test plants were rubbed with juice from diseased plants in the presence of carborundum powder and when razor blade cuts were made in the leaves through a drop or a film of infective sap. Transmission to Cymbidium occurred when pruning shears were used to cut into the leaves of healthy plants immediately after cutting the leaves of diseased plants, and even when infective juice was rubbed on the leaves with the fingers without an abrasive, The shortest time between inoculation and the appearance of symptoms was 4 weeks in both Cattleya and Cymbidium.

Murakishi (10) reported that Vanda mosaic virus was readily transmitted not only by the carborundum method but also by needle-pricks and by cuts made

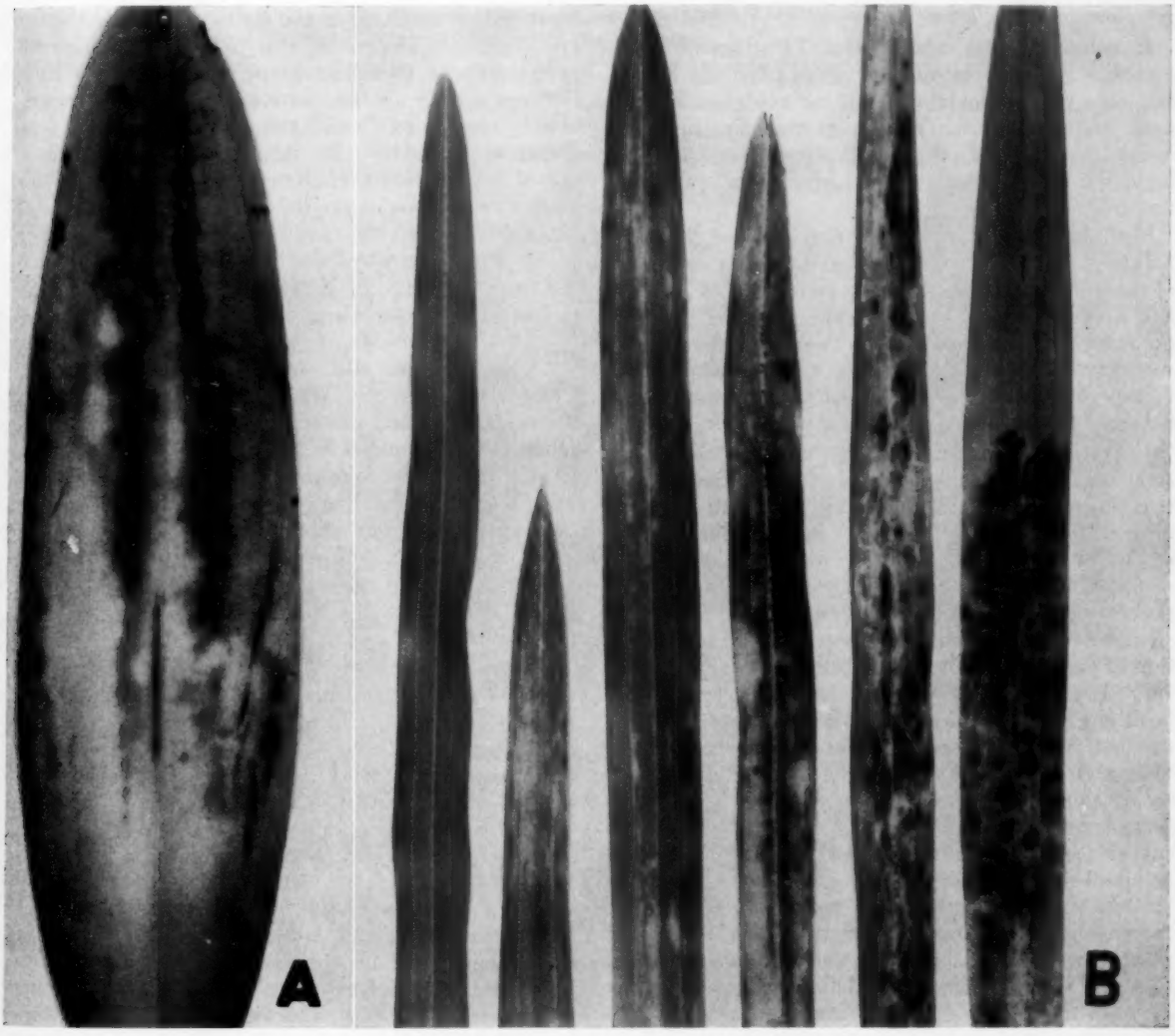

FIG. 2. Cymbidium mosaic virus symptoms in Cattleya and Cymbidium. A) Necrosis and chlorosis in lower surface of leaf on experimentally-infected Cattleya. B) Cymbidium leaves. Five leaves on the right show progression of symptoms from first diffuse mosaic through chlorosis and necrosis. Leaf at left is healthy.

with knives, shears, or other pruning tools rubbed with juice from diseased plants.

Cymbidium mosaic virus was transmitted from Cattleya and Cymbidium to 6 of 6 inoculated Cymbidiums and to 2 of 5 inoculated Cattleyas by means of razor cuts even though the Cymbidium tissue adjacent to the cuts died. Suberization developed around the cuts made in the Cattleya leaves. Ten of 11 Cymbidiums were inoculated successfully by cutting test leaves within 10 seconds after cutting diseased leaves or after contaminating the shears with infective juice. This high transfer followed leaf-cutting although extensive necrosis was apparent in the mechanically-injured areas shortly after cutting. Three Cattleyas, similarly inoculated, failed to develop disease symptoms.

A single Cymbidium seedling was inoculated by rubbing infective juice on the leaves with the fingers without any abrasive. This plant developed definite symptoms 6 weeks later.

Inoculations were made from 17 different virus sources. Because the number of healthy test plants available was unavoidably small for any given experiment and because the percentage transfers by all 4 methods of mechanical inoculation was unexpectedly high, the data do not prove which method or source was best.

The inocula used on the 65 Cattleya and 77 Cymbidium test plants came from the flowers or leaves of the orchid species *Cattleya trianaei* Lind. & Reichb. and *Epidendrum* spp. and from the following hybrids: *C. waltersiana*, unidentified Cattleya hybrids, *Brassocattleya dietrichiana*, *Laeliocattleya proca*, *L. pasadena*, *L. sargon*, *L. shoshone*, *L. lustre plumosa*, and Cymbidium hybrids.

Because some of the necrotic symptoms in Cattleya simulate those one might expect from pathogens, an experiment was carried out to determine the type of necrosis that would follow nonsterile mechanical injury of the leaves without virus. In particular, the test was to reveal whether or not secondary pathogens entered the wounded tissue and produced necrotic symptoms similar to those known to follow mechanical inoculation of virus. The leaves of 3 Cattleyas were similarly rubbed with distilled water and 3 with the juice of healthy Cymbidium leaves in the presence of carborundum. During the rubbing, the leaves were deliberately scratched to enhance the opportunity for necrosis to develop from causes other than virus, such as secondary fungus or bacterial invaders. Although the subsurface tissue in the vicinity of the lacerations acquired dark discoloration within a few minutes after the treatment, no further symptoms have developed in the ensuing 12 months. During this period other test plants from the same seed lot developed typical Cattleya necrosis after inoculation with juice from diseased Cattleya and Cymbidium plants.

Inoculations were made to 12 Cattleya plants from mosaic Cymbidium seedlings infected with virus from Cattleya and Laeliocattleya source plants. Seven of the 12 test Cattleyas developed symptoms. Moreover, 6 of 10 Cattleya seedlings developed necrosis after inoculation with virus from a standard Cymbidium mosaic source in Cymbidium. These tests confirm the assumption that the mosaic symptoms in Cymbidium were caused by the same virus that produced necrotic patterns in Cattleya leaves.

Cymbidium appears to be better than Cattleya as an indicator plant for this virus, since only 30 of 65 inoculated Cattleyas developed symptoms compared with 61 of 77 Cymbidiums. Moreover, many Cattleyas may show few, if any, symptoms after recovering from the initial shock of infection, whereas Cymbidium continues indefinitely to develop distinct mosaic symptoms, particularly in the new shoots. Also, the mottle symptoms in Cymbidium are more distinctly virus-like than are the necrotic symptoms in Cattleya.

HOST RANGE. — *Orchidaceae.* — Cymbidium mosaic virus is very common in Cymbidium and occurs to a lesser extent in Cattleya and its bigeneric and trigeneric hybrids. The virus also has been transmitted experimentally from species of *Epidendrum* to both Cattleya and Cymbidium during this study. Symptoms closely resembling Cymbidium mosaic have been produced in Cymbidium by virus experimentally transmitted from *Oncidium* sp., *Zygopetalum* sp., *Angraecum eburneum* Lindl., and *Laelia anceps* Lindl. (6). Murakishi (11) transmitted a virus from mosaic Cymbidium leaves to Spathoglottis.

Solanaceae.—In 1951, Jensen (5) reported that Cymbidium mosaic virus failed to infect plants of *Nicotiana tabacum* L., *N. glutinosa* L., and *Lycopersicon esculentum* Mill. in the family Solanaceae. Recently Mr. N. H. White, University of Sidney, Australia, informed the writers by letter that he had found *Datura stramonium* L. susceptible to Cymbidium mosaic virus. The following statement is quoted with Mr. White's permission: "On *Datura*, local lesions developed on the inoculated leaves within 10 days after inoculation. These lesions resembled those caused by tobacco mosaic virus on *Datura*. The local lesion development was not followed by any systemic symptoms."

Using virus from both mosaic Cymbidium and necrosed Cattleya, we have confirmed White's results for *D. stramonium* but have obtained no symptoms on 5 plants of *D. meteloides* Dunal. This observation emphasizes the limited host range of the virus. In our experiments with *D. stramonium*, the lesions developed first on the oldest inoculated leaves (14 days after inoculation) and last on the youngest inoculated leaves (24 days). The course of lesion development in the plant suggests that a certain maturity in the leaf tissue is essential for symptom expression. The first lesions could be discerned on the distal portions of the oldest inoculated leaves in 13–14 days. During the following 2–3 days, additional lesions appeared on the middle portion of these leaves and on the distal portion of the next youngest leaves. Lesions continued to develop for 7–10 days, the most recent lesions

always appearing on progressively younger inoculated leaves. Fewer lesions developed on the young leaves than on the old leaves.

The lesions appear first as small round brown spots (Fig. 3, A). Within 3 days the leaf tissue around the lesion becomes water-soaked (Fig. 3, B) and subsequently dies and becomes dry. The leaf tissue between the water-soaked areas becomes chlorotic (Fig. 3, C), and the leaf drops prematurely.

The lesions of Cymbidium mosaic virus in Datura resemble in some respects those caused by tobacco mosaic virus (Fig. 3, E), but there are several differences. The latter lesions can be detected within 2 days after inoculation and are well defined in 3 days, while those caused by Cymbidium mosaic virus require 14 days or more for development. Tobacco mosaic lesions appear in the young leaves about as soon as they do in the older leaves, whereas the incubation period of Cymbidium mosaic lesions is inversely proportional to the age of the leaf at the time of inoculation. Tobacco mosaic lesions remain relatively discrete, and affected leaves lack the extensive water-soaked areas and the tendency to drop prematurely characteristic of leaves infected with Cymbidium mosaic virus.

Thirty plants of *D. stramonium* were inoculated mechanically with juice from mosaic Cymbidiums and 3 plants were inoculated with juice from necrosed Cattleya. All test plants developed lesions on the inoculated leaves. Some of these plants were inoculated a second time, 3 weeks after the first inoculation, on both young and older leaves which had not been inoculated previously. These leaves also developed typical symptoms.

Juice extracted from lesion-bearing Datura leaves 21 days after inoculation with Cymbidium mosaic virus failed to cause infections in any of 5 Cymbidium seedlings, but it produced typical lesions in the leaves of 4 Datura plants.

Lesion-bearing leaf tissue of *D. stramonium*, examined in the electron microscope, was found to contain rod-shaped particles indistinguishable from those associated with Cymbidium mosaic virus in Cymbidium and Cattleya.

ELECTRON MICROSCOPY SHOWS IDENTITY OF VIRUS.—

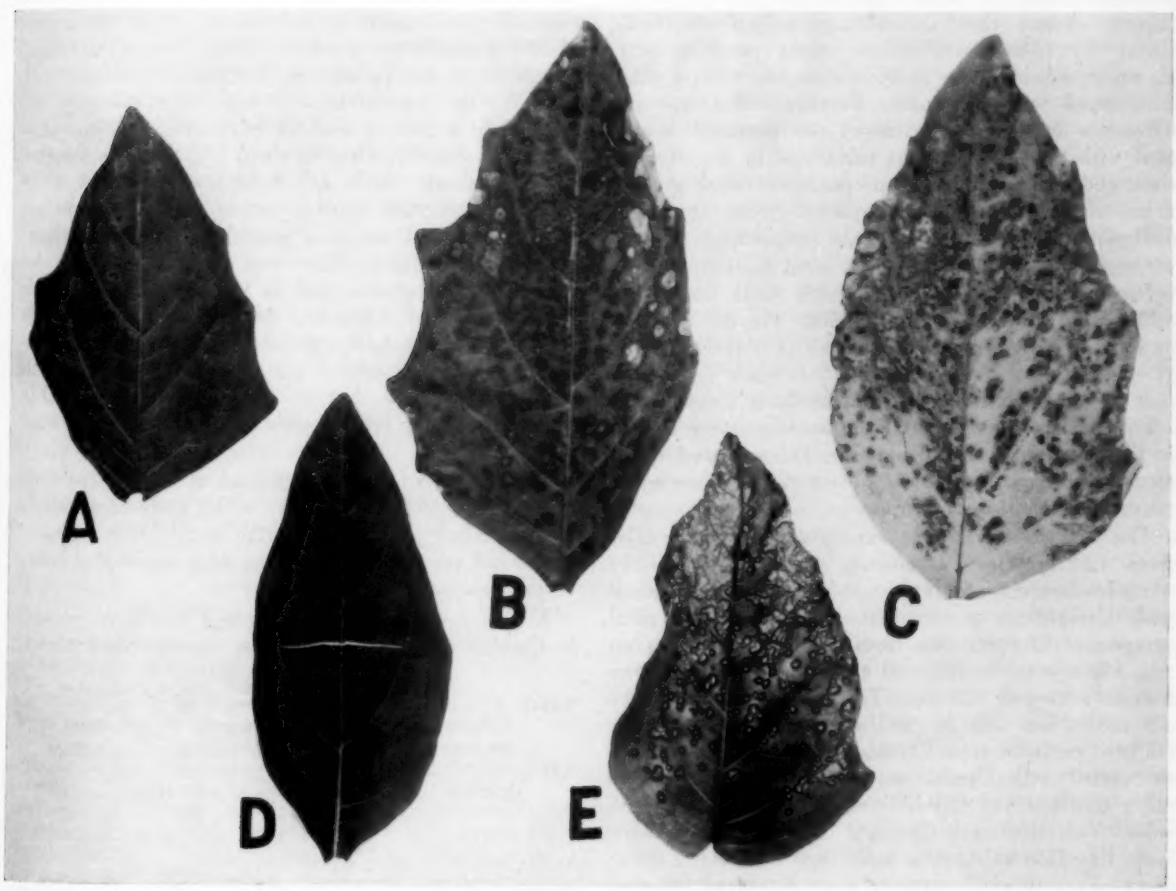

FIG. 3. Symptoms of Cymbidium mosaic virus (A-C) and tobacco mosaic virus (E) on leaves of *Datura stramonium*. A) Immature leaf showing new lesions. B) Older leaf showing water-soaked areas around lesions. C) Older leaf showing chlorosis of leaf and drying of tissue around lesions. D) Healthy leaf. E) Lesions of tobacco mosaic virus 13 days after inoculation.

The shape, size, and concentration of a particle type associated with Cymbidium mosaic virus in Cattleya and Cymbidium were determined by electron microscopy.

The methods used to concentrate and purify the particles were essentially those described before (2), involving a series of cycles of low- and high-speed centrifugation. The methods used in preparing samples were also the same as those previously described, except that specimens were shadowed with uranium instead of with palladium. The purified virus pellets from both Cattleya and Cymbidium were transparent and colorless. A pellet of the purified virus suspended in water produced characteristic symptoms in 3 of 4 Cymbidiums.

Preparations from naturally-infected Cattleya and from experimentally-infected Cymbidium (Fig. 3) were found to contain flexuous particles similar in shape and size to those previously described for Cymbidium mosaic virus (2) and clearly distinct from the shorter, thicker, more rigid particles associated with Odontoglossum ringspot virus (7).

Preliminary measurements suggested that the particles associated with Cattleya necrosis might be slightly shorter than the average length previously found for Cymbidium mosaic virus particles (2). However, measurement of approximately 950 particles established with reasonable certainty that no real difference exists in the size of the particles associated with the 2 hosts. But variations in the average sizes obtained in different preparations were greater than would have been predicted from statistical analysis of results from a single preparation.

Samples from leaves were prepared for examination by grinding 1 g of the tissue in a little water and diluting to a final dilution of 1-500. For the determination of particle concentration, polystyrene latex (Dow 580G lot 10 t) was added in such proportion that the final concentration of the latex spheres was 1.6×10^{10} spheres per ml. The homogenate prepared in this way was sprayed on to collodion-coated specimen screens and shadowed. Measurements were made on projections of micrographs.

The first measurements were made of 84 particles from Cattleya leaves showing necrosis and of 99 particles from Cymbidium leaves known to be infected with Cymbidium mosaic virus and showing typical symptoms. The particles from necrotic Cattleya average 448 mμ in length, and those from mosaic Cymbidium averaged 488 mμ. To determine whether the difference was due to the host, measurements were made of particles from Cymbidium and Cattleya plants inoculated with Cymbidium mosaic virus, and from other Cattleya and Cymbidium plants inoculated with juice from necrosed Cattleya. These measurements were based on calibration with Dow 580 G (lot 10 t) latex. From 68–117 particles were measured for each treatment.

The average particle lengths for the various preparations did not differ significantly. However, the lengths appeared to be consistently shorter than had previously been observed for Cymbidium mosaic virus (2). Tests indicated that the latex particles used for size calibration had changed slightly in size during storage. Therefore a final comparison of particle size was made from leaves of Cymbidium inoculated with Cymbidium mosaic virus and from leaves of Cymbidium inoculated with juice from necrotic Cattleya leaves. The added latex used as a standard was either of 2 lots, Dow Physical Research Laboratory No. 15N-7 and 15N-8. The particles in these lots had been determined as 0.333 mμ and 0.514 mμ in diameter, respectively.

There was no significant difference in length of the particles associated with the 2 diseases (Table 1). The apparent size in these tests was in good agreement with that observed earlier (2) in tests where latex 580G (lot 10 t) was used.

Virus concentration.—Assays of 3 samples of Cymbidium mosaic virus from Cymbidium were performed according to the method of Backus and Williams (1). The indicated concentrations of particles were 8.5×10^{12}, 1.5×10^{13}, and 1.6×10^{13} particles per g fresh weight. The highest concentrations of tobacco mosaic particles in tobacco are of the order of 10^{14} particles per g fresh weight. Thus Cymbidium mosaic virus was present in Cattleya to the extent of 1/10–1/5 the concentration of tobacco mosaic virus in tobacco in terms of number of particles. Since the Cymbidium mosaic virus is about 1½ times as long as tobacco mosaic virus, the relative concentration of Cymbidium mosaic virus is probably even greater on a weight basis than on a particle-count basis. Cymbidium mosaic virus thus must be considered to be rather highly concentrated in the host cells of both Cymbidium and Cattleya. In this characteristic it also resembles tobacco mosaic virus, which is commonly recognized as being present in tissue in such high concentration that it constitutes a considerable proportion of the total protein in systemically infected plants.

Control.—No method is known at the present time whereby orchid plants infected with Cymbidium mosaic can be freed of virus. Routine procedures followed by orchid growers may well be responsible for many of the new cases of infection.

A survey made in California of Cymbidium mosaic in Cymbidium (5) revealed that the disease occurred

Table 1.—*Comparative particle length of virus from Cymbidium inoculated from mosaic Cymbidium and necrotic Cattleya*

Original virus source	Number particles measured	Average particle length	Polystyrene lot
		mμ	
Mosaic Cymbidium	94	480	15N-8
Mosaic Cymbidium	121	474	15N-7
Necrotic Cattleya	91	492	15N-8
Necrotic Cattleya	76	462	15N-7

almost exclusively in plants which were over 10 years of age. No disease was found in younger hybrid seedlings even when these were grown for several years in lath-house beds in close proximity to older diseased plants. This circumstance suggests that if insects are important in the transmission of Cymbidium mosaic virus, such spread does not occur until after the plants have been of a flowering age for several years. Thus far insects have not been demonstrated to carry this virus.

Spread of the virus by mechanical means is a more plausible explanation for the high incidence of mosaic among the older commercial hybrids. Most hybrid seedlings are unexposed to this type of virus spread until they are several years old (large enough to flower and ready to be divided). The ease with which Cymbidium mosaic virus is transmitted experimentally from diseased to healthy orchids by pruning shears, by razor-blade cuts, and by workers' hands indicates strongly that contaminated cutting tools may be responsible for many, if not most, of the new infections. The tools can become contaminated with virus during the division of plants, the harvest of flowers, and the cutting of leaves.

Preliminary trials of dipping cutting instruments into plain water and into water containing a detergent were disappointing. A dangerous amont of infection followed the use of tools so treated (Table 2). Until a suitable dip is devised for cutting instruments, growers are advised to segregate healthy plants from the diseased ones when the plants are being divided and when the flowers are being harvested.

Discussion.—Cattleya necrosis is clearly distinct in its symptoms from other virus diseases recorded in the literature except that reported from Hawaii (9) and the disease caused by Cymbidium necrotic ringspot virus in Cattleya (6). The latter virus causes severe necrosis of the young shoots as well as of the older leaves.

The incidence of Cymbidium mosaic virus in plantings of Cattleya is likely to be much higher than that suggested by the number of plants showing necrotic symptoms at any given time. Many plants infected with virus do not show symptoms all of the time. Symptom expression appears to be irregular and, in general, milder after the plant goes through the shock effect that frequently follows infection. Failure to develop symptoms or loss of symptom-bearing leaves by diseased plants is the probable explanation for the finding of rod-shaped particles in the purified extracts of 4 out of 8 apparently healthy Cattleya orchid plants tested by Newton and Rosberg (12).

Table 2.—*Transmission of Cymbidium mosaic virus to Cymbidium seedlings by means of pruning shears contaminated with infective juice and dipped in water or water plus detergent*

Treatment[a]	Plants tested	Plants infected
Infective juice only	11	10
Infective juice only; cuts extended across entire test leaf[b]	11	6
Blades dipped into distilled water before cutting test plant	10	5
Blades dipped into a detergent-water solution before cutting test plant	10	4

[a] Approximately 20 cuts were made into leaves of each test plant. Except as indicated, cuts did not extend beyond the midrib.

[b] Cuts extended across the entire leaf and resulted in the death of many leaves beyond the basal cut.

The problem of diagnosis in Cattleya is complicated also by the failure of Cymbidium mosaic virus to cause variegation in Cattleya flowers. In Cymbidium, although the flowers also remain symptomless, the virus consistently induces well-defined mosaic symptoms in the leaves. Cymbidium very rarely, if ever, fails to show mosaic symptoms in the new shoots produced after infection.

Earlier reports (3, 8) stating that some of the flower breaking in Cattleya appeared to be associated with the flexuous particles of Cymbidium mosaic virus were incorrect. The present investigation has shown that the sinuous virus particles which cause mosaic in Cymbidium and leaf necrosis in Cattleya produce no color breaking in the flowers of Cattleya or of Cymbidium. Consequently, we must conclude either 1) that the variegated Cattleya flowers used as inoculum source in some of the transmissions to Cymbidium carried Cymbidium mosaic virus symptomlessly in addition to a flower breaking virus, or 2) that the particles of the virus causing some of the flower variegation in Cattleya are indistinguishable morphologically from those of Cymbidium mosaic virus and that this flower-breaking virus also causes mosaic symptoms in Cymbidium indistinguishable from common Cymbidium mosaic. The evidence favors the first alternative.

Although 10 different necrotic Cattleya, Brassocattleya, and Laeliocattleya plants selected from commercial greenhouses apparently carried Cymbidium mosaic virus, similar necrotic symptoms can be caused by at least 1 other virus. The virus of the disease described as Cymbidium necrotic ringspot (6) produces symptoms in Cymbidium quite different from Cymbidium mosaic. However, when inoculated into Cattleya, this virus causes a disease that in many plants could not be distinguished from the Cattleya necrosis caused by Cymbidium mosaic virus. In Cattleya, however, the ringspot virus typically necroses both young and old leaves, thus differing from the Cymbidium mosaic virus that usually necroses only the old leaves. Moreover, the ringspot virus affects Cattleya so severely that it is apparently self-eliminating to a considerable degree, either by killing the entire plant or so much of it that the plant is discarded by the grower.

The incidence of Cymbidium mosaic virus in both Cymbidium and Cattleya in commercial greenhouses

has been much higher than seems reasonably explicable by the assumption that some insect vector causes most of the spread to previously healthy plants. Insect control in most establishments has been good and attempts have failed to transmit the virus experimentally by means of insects. Our present demonstration that the virus can be transmitted easily both by pruning shears and by razor cuts provides strong circumstantial evidence that the virus may be spreading from plant to plant on contaminated knives and other tools used in cutting flowers, in pruning off dead portions of leaves, and in dividing and repotting plants.

Datura stramonium, shown to be susceptible to Cymbidium mosaic virus, is the first known nonorchidaceous host of the virus. Since the virus causes local lesions in the leaves 2 weeks after inoculation, *D. stramonium* should be very useful in orchid virus work and permit an inexpensive and relatively rapid test for Cymbidium mosaic virus.

Departments of Entomology and Plant Pathology
University of California
Berkeley, California

LITERATURE CITED

1. Backus, R. C., and R. C. Williams. 1949. The use of spraying methods and volatile suspension media in the preparation of specimens for electron microscopy. Jour. Appl. Phys. **21**: 11-15.
2. Gold, A. H., and D. D. Jensen. 1951. An electron microscope study of Cymbidium mosaic virus. Amer. Jour. Bot. **38**: 577-578.
3. Gold, A. H., and D. D. Jensen. 1952. Some apparent virus relationships in several orchid genera, based on electron microscopy. Phytopathology **42**: 9.
4. Jensen, D. D. 1950. Mosaic of Cymbidium orchids. Phytopathology **40**: 966-967.
5. Jensen, D. D. 1951. Mosaic or black streak disease of Cymbidium orchids. Phytopathology **41**: 401-414.
6. Jensen, D. D. 1953. Virus diseases of Cymbidiums. Amer. Orchid Soc. Bul. **22**: 800-804.
7. Jensen, D. D., and A. H. Gold. 1951. A virus ringspot of Odontoglossum orchid: symptoms, transmission, and electron microscopy. Phytopathology **41**: 648-653.
8. Jensen, D. D., and A. H. Gold. 1952. Virus diseases of orchids in California. Phytopathology **42**: 12.
9. Murakishi, H. H. 1952. Two new virus diseases in Hawaii. Phytopathology **42**: 517.
10. Murakishi, H. H. 1953. Vanda mosaic can be controlled. Hawaii Farm Sci. **1**(4): 1, 6.
11. Murakishi, H. H. 1954. Spathoglottis, a good indicator plant for orchid viruses. Bul. Pac. Orchid Soc. Hawaii **12**: 20-22.
12. Newton, N., and D. W. Rosberg. 1952. Electron-microscope studies of a new orchid virus complex. Phytopathology **42**: 79-82.

BIOPHYSICAL CHARACTERISTICS OF THE TOMATO-RINGSPOT VIRUS[1]

Robert P. Kahn, Paul R. Desjardins,[2] and C. A. Senseney

SUMMARY

Preparations from tobacco plants infected by tomato-ringspot virus and from plants free of virus were subjected to differential centrifugation in the preparative centrifuge and then to electrophoretic fractionation. Four components were apparent in preparations from virus-infected tissue following 60 minutes of electrophoresis whereas only 1 component was detected in preparations from healthy plants. Five fractions were separated after 312 minutes of electrophoresis. Infectivity tests and optical density determinations at 260 mμ for each of these 5 fractions indicate that the virus, or nucleoprotein, was associated with the slowest moving of the 4 components. The mobility of that component was 2.4×10^{-5} cm^2 volt^{-1} sec^{-1}. The correlation coefficient between local-lesion counts on cowpea plants and particle counts on electron micrographs was 0.997, indicating that infectivity was associated with the type of particles counted.

The tomato-ringspot virus resembles the tobacco-ringspot virus in its host range (6) and thermal inactivation point (5). However, these 2 viruses have been differentiated on the basis of cross-immunity tests and serological reactions (11) and by particle size and shape (7). The biophysical characteristics of the tobacco-ringspot virus have been described previously from this laboratory (2). Apparently no such studies have appeared in the literature for the tomato-ringspot virus. These investigations of the tomato-ringspot virus were initiated to complete comparative studies of these 2 viruses along biophysical lines and to attempt

[1] Accepted for publication February 6, 1955.
[2] Present address, Department of Plant Pathology, University of California, Riverside, California.

Grateful acknowledgement is extended by the authors to Drs. J. E. Mitchell and R. W. Fulton for their constructive reviews of this paper, to Dr. W. C. Price for supplying the virus used, and to Dr. J. Wagman, Mr. George Hess, and Mr. J. H. Convey for assistance with the biophysical tests.

to correlate electron-micrograph particle-counts with infectivity.

METHODS AND MATERIALS.—The Price isolate of the tomato-ringspot virus (tobacco-ringspot virus No. 2) was used in these experiments. Cross-immunity tests employing rooted stem-tips from tobacco plants recovered from either the tomato- or the tobacco-ringspot virus indicated that the Price isolate of the tomato-ringspot virus was distinct from the tobacco-ringspot isolate used in the previously reported investigations (2).

Tomato-ringspot virus was produced in Holmes' necrotic-type tobacco and purified by alternate low- and high-speed centrifugation and by electrophoretic fractionation. Leaves from infected tobacco plants showing typical symptoms and comparable leaves from virus-free plants were minced. Sufficient dipotassium phosphate solution was added to bring the final concentration of this salt to 3 per cent by weight of the minced tissue. The juice was expressed from the pulp with a Carver press and clarified by low-speed centrifugation. Initial concentration and purification were achieved by differential centrifugation in the preparative centrifuge, Spinco Model L, rotor No. 30 (8, 9, 10). In accordance with this procedure, the pellets obtained from each high-speed run for both diseased and healthy tissue were suspended in 0.01 M potassium phosphate buffer at pH 7 for each subsequent low-speed run. Pellets from the final high-speed centrifugation were resuspended in 0.1 ionic strength potassium phosphate buffer at pH 6.5 for use in the electrophoresis apparatus.

Electrophoresis.—Pellets from the final high-speed centrifugation in the preparative centrifuge were subjected to electrophoresis in the Tiselius apparatus. The specific conductance was 1.25×10^{-4} mho with a 10-ma current. The temperature was 1.3°C. The protein concentration of preparations from infected plants was 0.23 per cent, while that from healthy plants was 0.11 per cent as determined by refractive indices. At the end of 60 minutes, electrophoretic mobilities were determined. After 312 minutes, fractions were separated from the ascending and from the descending boundaries for use in optical density determinations, in the bioassay, and for electron microscopy.

Ultraviolet absorption.—The Beckman UV spectrophotometer was utilized to determine the ultraviolet absorption spectra of resuspended pellets from the final high-speed centrifugation in the ultra-centrifuge. Preparations from virus-free plants and from virus-infected plants were tested in a wavelength range of 220 to 290 mμ.

Analytical centrifugation.—Sedimentation constants of particles in the supernatants of a final low-speed centrifugation in the preparative centrifuge were determined in the Spinco analytical centrifuge. A dilution of 1:500 in 0.062M phosphate buffer was used and the solution was centrifuged for 60 minutes at 39,000 rpm.

Electron microscopy.—Specimens for electron microscopy were prepared by the protein monolayer technique (3), shadowed with uranium, and examined in an RCA type EMU microscope. The protein monolayer technique involved the application of a suspension of virus particles in a dilute solution of bovine plasma albumen to a point on an aqueous surface. This protein spread spontaneously to form an insoluble monolayer in which the particles to be examined were uniformly distributed. The monolayer with its imbedded particles was transferred to a collodion-covered screen and shadowed with 8 A of uranium at a grazing angle of 16 degrees. The objective pole piece of the electron microscope was corrected according to Hillier and Ramberg (4). A self-biased gun was used as the electron source. The magnification was calibrated by using a replica of a precision-ruled grating manufactured by the Ford Company which contained 15,000 lines/in. This procedure was employed in obtaining the previously published electron micrographs of tomato-ringspot virus (7) and tobacco-ringspot virus (2).

Bioassay.—The relative infectivities of virus preparations used in these investigations were ascertained by bioassay on the Wilt Resistant or Black varieties of cowpeas, *Vigna sinensis* Endl. Plants were inoculated by rubbing Celite-dusted primary leaves with a ground-glass spatula previously dipped in virus inoculum. Leaves were rinsed with tap water after inoculation. Local lesions were counted after a 4- to 5-day incubation period in the greenhouse.

EXPERIMENTAL RESULTS.—*Electrophoretic fractionation.*—Four distinct protein components were apparent in the preparations obtained from virus-infected tissues following 60 minutes of electrophoresis. This contrasted sharply with the situation in the preparations from healthy tissue where only 1 component was detected. The mobility of this 1 component was 6.87×10^{-5} cm^2 volt^{-1} sec^{-1} for the ascending boundary and 6.46×10^{-5} cm^2 volt^{-1} sec^{-1} for the descending boundary. The characteristics of the components in preparations from infected tissue are shown in Table 1 and Figure 1. From these data, it may be seen that the mobility of the component 3 (6.76×10^{-5} cm^2 volt^{-1} sec^{-1}) was similar to that of the 1 component in preparations from virus-free tissue.

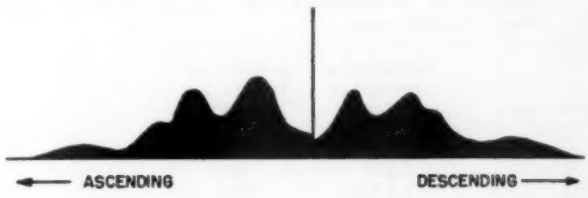

FIG. 1. Electrophoretic patterns of preparations from plants infected by tomato-ringspot virus (after 60 minutes in the Tiselius apparatus).

TABLE 1.—*Electrophoretic mobilities of various components of tomato-ringspot virus preparation*

Ascending			Descending		
Component	μ[a]	Conc.	Component	μ[a]	Conc.
		per cent			per cent
1	—16.97	8.4	1	—15.85	7.8
2	— 9.95	13.3	2	— 8.51	20.3
3	— 6.88	37.4	3	— 6.46	37.5
4	— 2.14	40.9	4	— 2.00	34.4

[a] μ is $10^5 \times$ mobility in cm^2 $volt^{-1}$ sec^{-1}

Five fractions were separated after 312 minutes of electrophoresis in the Tiselius apparatus. The infectivity, optical density, and components of each fraction are shown in Table 2. Fraction I, containing components 1 and 2, and Fraction II containing components 1, 2, and 3 were only slightly infective as determined by the bioassay. These fractions contained relatively little nucleoprotein as determined by optical density at 260 mμ. Thus, the virus, or nucleoprotein, was not apparently associated with components 1, 2, or 3. On the other hand, Fraction III, containing components 1, 2, 3, and 4, Fraction IV containing components 3 and 4, and Fraction V containing component 4 all had high infectivity titers and high optical densities, indicating the presence of the virus and of nucleoprotein. The low infectivity and optical density exhibited by components 1, 2, and 3, and the high infectivity and optical density shown by combinations of 1, 2, 3, and 4, and of 3 and 4, and by 4 alone indicate that infectivity and high nucleoprotein concentrations are associated with component 4.

Particle counts on electron micrographs as related to infectivity.—Particle counts from electron-micrographs of preparations from electrophoretic Fraction V were compared with local-lesion counts obtained from cowpeas inoculated with aliquots of these preparations. These counts were obtained for both the supernatant and the resuspended pellets for each of 3 sedimentation periods in the preparative centrifuge at 40,000 rpm (Table 3). The previously published (7) electron micrograph of preparations from tobacco plants infected with the tomato-ringspot virus as reprinted in Figure 2 was representative of those micrographs used for particle counts.

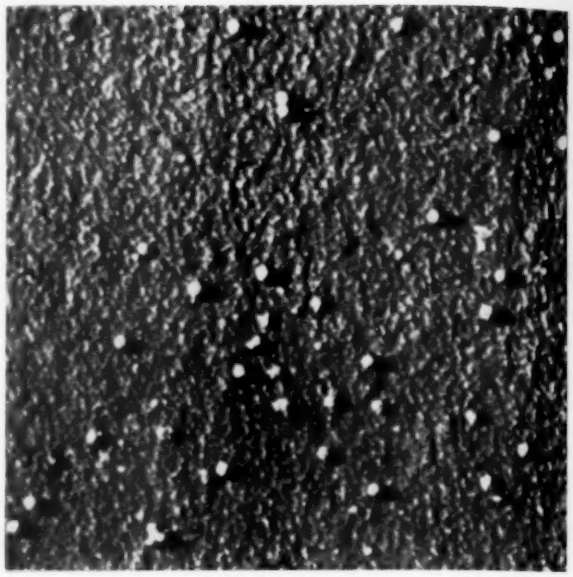

FIG. 2. Electron micrograph of particles from purified tomato-ringspot virus preparations ($\times$ 54,000).

The data in Table 3 when subjected to the Z transformation show a correlation coefficient of 0.997 between particle counts and lesion counts. Visual inspection of the data shows that as the sedimentation time increased, the infectivity and particle count of the supernatant decreased. At the same time infectivity and particle counts of solutions of the pellets increased.

Ultraviolet absorption.—Preparations from infective material showed a more pronounced maximum at 260 mμ than did preparations from virus-free material. Thus, a high concentration of nucleoprotein was associated with preparations from virus-infected plants.

Sedimentation constant.—Three boundaries for preparations from virus-infected tobacco tissue and

TABLE 2.—*Infectivity and optical density of electrophoretic fractions of preparations from plants infected by tomato-ringspot virus*

Fraction	Components present in fraction	Average no. lesions per leaf[a]	Optical density at 260 mμ
I	1,2	1.4	0.043
II	1,2,3	29.0	0.082
III	1,2,3,4,	182.3	0.223
IV	3,4	204.6	0.216
V	4	176.9	0.198

[a] Ten leaves used in each test.

TABLE 3.—*Infectivity measurements and particle counts at intervals during sedimentation of a highly infectious electrophoretic fraction (Fraction V)*

Duration of centrifuging (minutes)	Total number of lesions[a]		Average electron-microscope particle-count[b] per observed area	
	Pellet	Supernatant	Pellet	Supernatant
7	1100	501	594	282
21	1518	24	879	10
63	1998	1	882	4

[a] 20 plants inoculated for each time period.
[b] 25 areas counted each with an area of 10×10^{-7} cm^2.

1 from healthy tissue were indicated by the analytical centrifugation. The single boundary from healthy-tissue preparations appeared early during the acceleration and consisted of a heavy diffuse material associated with the amber color of the extract. The 3 boundaries from the infected tissue preparations consisted of: 1) the fastest moving boundary, accounting for 54 per cent of the material and having a sedimentation constant at 20°C (Sw 20 value) of 122; 2) a boundary accounting for 18 per cent of the material and with a Sw 20 value of 91; and 3) a boundary comprising 28 per cent with a Sw value of 55. The relative infectivities of the materials represented by the 3 boundaries were not determined since a separation cell was not available.

DISCUSSION.—At the beginning of these biophysical tests, it was assumed on the basis of the absence of symptoms that the noninoculated tobacco plants were free of virus. At the conclusion of these tests, it was evident that such an assumption was valid since preparations from the noninoculated plants were not infective, showed little absorption at 260 mμ, and were free of "virus-like particles" upon examination with the electron microscope. Since the ringspot symptoms in the inoculated plants could have represented either the tomato-ringspot virus or the tobacco-ringspot virus, it was necessary to establish by cross-immunity tests that the virus isolate employed in these tomato-ringspot experiments was distinct from the isolate used in the previously reported tobacco-ringspot virus experiments (2).

Although the size and shape of particles on electron micrographs as reported previously (2, 7) served to differentiate these 2 viruses, the other biophysical tests reported herein revealed similarities. For both tobacco-ringspot virus (2) and tomato-ringspot virus, electrophoresis indicated 4 components for preparations from virus-infected plants and 1 component for preparations from plants free of virus. Infectivity tests indicated that the viruses were associated with the slowest-moving component. Similarly, optical density tests at 260 mμ showed that high nucleoprotein concentrations were present in the same slow-moving component. The mobility of this slow-moving component in the ascending column for tomato-ringspot preparations was 2.14×10^{-5} cm^2 volt^{-1} sec^{-1} while the value for tobacco-ringspot virus as reported previously was 1.39×10^{-5} cm^2 volt^{-1} sec^{-1}. Both viruses showed a high correlation coefficient between particle counts on electron micrographs and lesion counts on cowpea leaves. The coefficient for tomato-ringspot virus was 0.997 while that for tobacco-ringspot virus was 0.970.

The molecular weight of the tomato-ringspot virus is estimated to be 7,800,000 based on a sedimentation constant of 122, particle dimensions of 43.0 × 13.5 mμ (7) and an assumed density of 1.35.

CAMP DETRICK
FREDERICK, MARYLAND

LITERATURE CITED

1. BAWDEN, F. C. 1950. Plant viruses and virus diseases. Ed. 3. Chronica Botanica Co., Waltham, Mass. 335 p.
2. DESJARDINS, P. R., C. A. SENSENEY, AND G. E. HESS. 1953. Further studies on the electron microscopy of purified tobacco ringspot virus. Phytopathology **43**:687-690.
3. HARTMAN, R. E., T. D. GREEN, J. B. BATEMAN, C. A. SENSENEY, AND G. E. HESS. 1953. Preparation of uniformly dispersed specimens of particulate matter for microscopy. Jour. Appl. Phys. **24**:90-92.
4. HILLIER, J., AND G. E. RAMBERG. 1947. The magnetic electron microscope objective; contour phenomenon and the attainment of high resolving power. Jour. Appl. Phys. **18**:48-71.
5. HOLMES, F. O. The filterable viruses. 1948. Williams and Wilkens. Baltimore. 159 p.
6. PRICE, W. C. 1940. Comparative host ranges of six plant viruses. Amer. Jour. Bot. **27**:530-541.
7. SENSENEY, C. A., R. P. KAHN, AND P. R. DESJARDINS. 1954. Particle size and shape of purified tomato-ringspot virus. Science **120**:456-457.
8. SMITH, K. M. 1951. Recent advances in the study of plant viruses. Ed. 2. Blakiston, Philadelphia. 300 p.
9. STANLEY, W. M. 1939. The isolation and properties of tobacco ring spot virus. Jour. Biol. Chem. **129**: 405-428.
10. STANLEY, W. M., AND M. A. LAUFFER. 1948. Physical and chemical procedures. In T. C. Rivers, ed. Viral and reckittsial infection of man. Lippincott, Philadelphia, p. 18-59.
11. TALL, M. G., W. C. PRICE, AND K. WERTMAN. 1949. Differentiation of tobacco and tomato ringspot viruses by cross immunization and complement fixation. Phytopathology **39**:288-299.

FOLIAGE AS A SOURCE OF SECONDARY INOCULUM FOR TOMATO ANTHRACNOSE[1]

Maria E. Pantidou and W. T. Schroeder

SUMMARY

The observation that foliage of field-grown tomatoes harbored spores capable of producing tomato anthracnose prompted a study of the source of that inoculum and the importance it might play in the development of the fruit disease. Considerable variation was encountered among the fruit-rotting isolates used in their ability to infect and penetrate the root system, but none of them progressed far enough through the tissue to be of any importance insofar as above ground inoculum is concerned. Greenhouse experiments showed that leaf tissue could be penetrated within 24 hours and that the fungi could grow and sporulate abundantly, depending upon the maturity of the tissue and the length of the postinfection humidity period. Sporulation also occurred abundantly in lesions resulting from flea beetle or from Alternaria. With the aid of a marker strain infectious to uninjured fruit, it was proved conclusively that infected foliage provided sufficient inoculum to be of considerable importance in fruit infection.

Tomato anthracnose, generally attributed to the fungus *Colletotrichum phomoides* (Sacc.) Chester, is the major disease of tomato in New York State. It is primarily important as a disease of the ripe fruit. Under field conditions, tomato foliage shows no effects of the disease although foliage symptoms have been experimentally induced (4, 8).

It has been generally assumed that the causal organism overwinters in soil debris and that primary inoculum therefrom constitutes the principal source of fruit infections throughout the growing season. The recent work of Kendrick and Walker (4) supports this view.

Early in the present investigation it was found that leaves of field-grown tomato plants harbored Colletotrichum spores capable of causing anthracnose when sprayed on detached tomato fruits in the laboratory. The primary aims of this investigation were to determine the origin of such foliage infection and its importance in the development of fruit anthracnose.

METHODS.—The tomato variety Gem was used throughout this study. Greenhouse plants were grown in sterilized containers filled with either pasteurized soil or sterilized sand.

Root inoculations were made by dipping the roots in a water-agar suspension of spores and mycelial fragments of the respective organisms. Roots of control plants were dipped in sterile water-agar. Isolations from root and stem tissue were made by surface-sterilizing the plant material, aseptically stripping off the epidermis, and plating pieces of internal tissue on potato dextrose agar.

Foliage inoculations were performed by atomizing onto the leaves spores suspended in sterilized water. Unless otherwise stated, the plants were subjected to an atmosphere saturated with moisture 24 hours before and 48 hours after inoculation.

Healthy detached tomato fruits from greenhouse plants were used for fruit inoculations. None was visibly injured. Fruits were first washed with distilled water, then sprayed with a suspension of spores in sterilized water, and incubated in moist chambers for 48 hours at room temperature. Control fruits were sprayed with sterile water. Isolations from fruit lesions were made by stripping the epidermis from the previously sterilized fruit surface and transferring pieces of internal tissue, by means of a flamed scalpel, to agar plates. The pathogenicity of each isolate obtained from root, stem, or leaf tissues was determined by back-inoculations to healthy tomato fruits. The cultural characters of the reisolates from the anthracnose lesions that developed on the fruits were compared with those of the original cultures.

All original isolates used in these investigations were single-spored and tested for pathogenicity on uninjured green and red fruits (Table 1). All isolates, except 1 and 5 which were albino or near-albino strains, were pathogenic to uninjured fruits. Isolate 32, *Glomerella cingulata* (Ston.) Spauld. & Schrenk, was used as a marker strain. It has a Gloeosporium type of growth characterized by small spores, absence of setae, and other distinct cultural characters.

Leaf material was examined microscopically either by making smears or free-hand sections and mounting in lactophenol containing cotton blue. In studies on fungus penetration, the material was fixed in formalin-aceto-alcohol, sectioned at 10 μ and stained either by Stoughton's thionin-orange G method or in crystal violet-gold orange.

ROOT INFECTION.—During the course of these investigations, *Colletotrichum* spp. capable of causing fruit anthracnose were isolated several times from platings of vascular tissue of roots and stems made to recover Verticillium from wilted tomato plants. Infection of tomato roots grown in sand artificially infested with *C. phomoides* was reported by Kendrick and Walker (4). They observed small, inconspicuous necrotic flecks on the tap root and hypocotyl and com-

[1] Accepted for publication February 6, 1955. Approved by the Director for publication as Journal Paper No. 985, New York State Agricultural Experiment Station, Geneva, New York, January 7, 1955.

TABLE 1.—*Sources and description of the isolates of Colletotrichum and Glomerella used in these investigations*

Isolate number	Organism	Source	Type of 10-day old growth on potato dextrose agar	Spore size[a] in μ
1	*C. phomoides*	Tomato fruit, Sheridan, New York	Mycelium smooth with purplish tint, nearly albino	3–7 × 3
4	*C. phomoides*	Tomato fruit, Fredonia, New York	Mycelium fluffy, solid gray-brown	10–22 × 3
5	*C. phomoides*	Tomato fruit, Wisconsin Agr. Expt. Sta.	Light yellowish growth. Albino	14–23 × 3.5
6	*C. phomoides*	Tomato fruit, Brant, New York	Gray mycelium, encircled by ring of sclerotia	12–22 × 3
8	*C. phomoides*	Tomato fruit, Rochester, New York	Black sclerotial type	12–22 × 3
10	*C. phomoides*	Tomato fruit, Geneva, New York	Mycelium zoned with black, gray and white rings	12–18 × 3
32	*Glomerella cingulata*	Apple, California Spray-Chemical Co., New Jersey	White mycelium encircled by profuse pink masses of spores	5–15 × 3
42	*Colletotrichum* sp.	Internal tissue of tomato stem, Fredonia, New York	Mycelium purplish to orange: sclerotia submerged	15–22 × 3
44	*Colletotrichum* sp.	Squash, Geneva, New York	Mycelium dark, thick; black sclerotia	16–18 × 3
50	*Colletotrichum* sp.	Internal tissue of tomato stem, Geneva, New York	Mycelium dark with smooth surface	18–22 × 3
70	*Colletotrichum* sp.	Tomato fruit skin, Germany	Mycelium light gray	14–18 × 3.5
74	*Colletotrichum* sp.	Tomato roots, Maine	Mycelium black, smooth. Heavy sclerotial type	18–24 × 3.5

[a] Based on at least 100 spores.

plete decay of a few lateral roots, but made no attempts to reisolate the fungus. In England, Canada, Australia, and Eire, *S. atramentarium* (Berk. & Br.) Taub. has been reported pathogenic to tomato roots, causing lesions and a progressive decay of the cortex, leaving a brittle vascular cylinder (1, 2, 5, 6).

Three separate experiments were conducted in the greenhouse to determine whether the stem infections could have originated via the root and if the fungus, by further growth through the internal plant tissues, would produce fructifications on stem and leaf surfaces. In the first experiment, Colletotrichum isolates were used alone and in combination with the Verticillium fungus. It was presumed that infection by the latter organism might be a prerequisite to infection by Colletotrichum. Since, however, no differences in the number of isolations were obtained, Verticillium did not appear necessary for infection, and was not included in the last 2 experiments. The isolates used differed in their ability to infect tomato roots and stems. Isolate 6 was never recovered from inoculated plants; whereas isolates 10, 32, 42, 44, 50, and 74 were recovered frequently. Isolate 74, originally isolated from tomato roots and highly infectious to tomato fruits, was most frequently recovered from the root and stem internodes, and it was the only isolate which induced brown root lesions similar to those described for *C. atramentarium*. No effect on the aboveground plant parts was apparent. All reisolations from the roots and stems sporulated in culture and resembled the original cultures. All were pathogenic to fruit. Some of the isolates were recovered from as high as the fourth internode, but all attempts to detect the fungus in free-hand and microtome sections of stem tissue were unsuccessful. No growth of the fungus through the internal tissues to the plant surface was observed.

FOLIAGE INFECTION.—Infection of tomato foliage by *C. phomoides* has been a debatable subject for many years. Recently it was demonstrated experimentally that the fungus can infect the foliage (4, 8). Whether such infection occurs in nature and whether it is important in the life cycle of the pathogen have not been adequately investigated.

Tomato foliage from field plants as a reservoir of Colletotrichum spores.—From 1951 through 1953, leaf collections were made from field tomato vines at various intervals during the growing season. Two types of leaf samples were taken from each plant. The first contained leaves in contact with the soil. Many of the leaves in these samples were senescent or dead. The other samples were taken from higher branches with entirely green leaves. The leaves were washed lightly with running water to remove soil particles and debris, then crushed in distilled water, and the resultant suspension filtered through cheesecloth. Mounts from the filtrates under the microscope showed that Colletotrichum-like spores were present in most of the samples.

Tomato fruits atomized with the leaf filtrates developed typical anthracnose lesions in all tests (Table 2). None of the control fruits, sprayed with distilled water, developed anthracnose. Even leaves picked from plants that bore only green fruits, as in the samples of August 1, 1952 and July 25, 1953, harbored spores which caused anthracnose when sprayed on tomato fruits. The infectious filtrate of August 14, 1954 came from leaves on plants that were in flower but which had not developed any fruit at the time of sampling. In that instance, leaf infection, apparently from soil inoculum, was favored by rainfalls which totaled 2.32 in. during the interval between transplant-

TABLE 2.—*The pathogenicity of Colletotirichum-like spores found on samples of leaves collected from field-grown Gem tomato plants in the vicinity of Geneva, New York, 1951 through 1953*

Date of sampling	Source of spores			
	Leaves in contact with soil Number of fruits		Leaves not in contact with soil Number of fruits	
	Inoculated	Infected	Inoculated	Infected
Sept. 1, 1951[a]	10	6	12	4
Sept. 1, 1951[a]	8	4	11	2
Aug. 1, 1952	8	2		
Aug. 12, 1952	8	7	7	2
Aug. 18, 1952	6	5	5	1
Aug. 20, 1952	8	5		
July 25, 1953	8	4		
Aug. 14, 1953[b]	10	5		

[a] Fruits with anthracnose were present on vines.
[b] Plants were in floral stage, no fruit was present.

ing, July 31, and sampling, August 14. Filtrates from leaves that touched the soil consistently gave higher percentages of infected fruits than those not in contact with the soil.

Early blight and flea beetle lesions as courts for anthracnose-causing fungi.—Two experiments were conducted to ascertain the extent of tomato foliage infection. The plants were grown in 8-in. pots of pasteurized soil. The soil surface was covered with vermiculite and the plants were staked to prevent soil contact. In the first experiment, the leaves of the plants, at the fourth to fifth node stage, were inoculated with spore suspensions of isolates 4, 6, 8, 10, 32, 42, 44, 50, 70, and 74. Eight single-plant replicates were inoculated with each isolate and 8 plants served as controls.

Many of the experimental plants were infected with Alternaria and almost all were injured by flea beetles at the time of inoculation. Two weeks after inoculation the plants were subjected to high humidity for 48 hours. A week later distinct anthracnose lesions were found only on the necrotic tips of the leaves (Fig. 1 A). Spores and setae were found in most of the early-blight and flea-beetle lesions examined (Fig. 1, B). Only isolate 32 produced spores without setae. Pieces of tissue from the necrotic tips yielded pure cultures of the fungi pathogenic to tomato fruits.

In the second experiment, only isolates 4 and 10 were used and the inoculated plants were subjected either to 48 or 72 hours postinoculation high humidity. As in the first experiment, several plants showed early-blight or flea-beetle lesions, and the isolates with which they were inoculated grew and sporulated in both. In addition, primary lesions similar to those described by Younkin and Dimock (8) and Kendrick and Walker (4) were observed. They were small, round chlorotic spots with pinpoint necrotic centers. Along the margins of the leaves the lesions were irregularly necrotic. Such primary lesions, however, were not abundant, were barely conspicuous, and exhibited few, if any, spores. Such lesions also developed under average greenhouse humidity conditions, but they did not develop further, even when the leaves matured. The postinoculation exposure to high humidity caused the areas around the necrotic spots to become brown and dead; acervuli were produced.

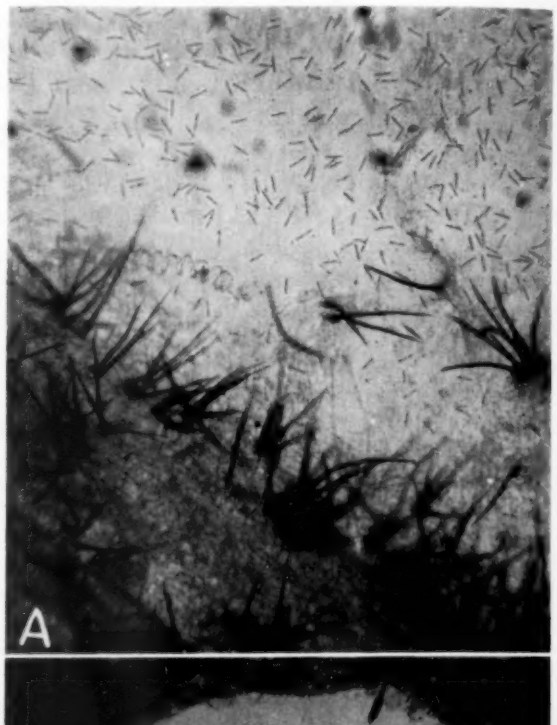

FIG. 1. Spores and setae of *C. phomoides* that developed in leaf lesions of Gem tomato following infection by isolate 4. A) Leaf-tip lesion. 200×. B) Flea-beetle lesion. 60×.

Sporulation and development of setae were proportional to the length of the postinoculation exposure to high humidity. Sporulation was abundant in the lower, more mature leaves. Stem lesions were few, appearing as small elongated spots. Some flowers were killed and the fungi sporulated abundantly when the flowers were subjected later to high humidity. All types of leaf and stem tissue were cultured and the reisolates proved pathogenic to tomato fruit.

After finding that anthracnose-causing fungi could grow and sporulate in early-blight and flea-beetle lesions under greenhouse conditions, efforts were directed during the summer of 1953 toward determining if such lesions in field-grown tomato plants harbored Colletotrichum.

Sixty leaves were picked on August 20 from plants which had been transplanted to the field at the end of July. They were surface sterilized and areas bearing flea-beetle lesions were plated out on potato dextrose agar. No fruits were present on the plants at the time the leaf samples were taken. Of the 60 plates, 2 yielded pure cultures of Colletotrichum which produced sclerotia, setae, and spores. In another 2 plates, Colletotrichum developed as sectors in plates bearing Alternaria. In a series of cultures made later in the season, 7 Colletotrichum cultures were obtained from 58 plated leaves. From another field, Colletotrichum was obtained in 5 of 68 plates cultured from flea-beetle-injured areas of leaves.

Attempts to recover the fungus from necrotic areas of leaves infected by Verticillium wilt organisms were unsuccessful.

Transmission of anthracnose-causing fungi from infected tomato foliage to green and red fruits.—Since it was demonstrated that anthracnose-causing fungi can grow and sporulate abundantly on the tomato foliage, the importance of this source of inoculum in causing fruit infection was determined in 3 greenhouse experiments.

Tomato seedlings, germinated and grown in sterilized quartz sand, were planted in sterilized 8-in. pots containing pasteurized soil. The surface of the soil in the pots was covered with vermiculite and the plants were staked at planting to prevent any contact with the soil. The leaves of the seedlings were inoculated when in the 2- to 3-node stage, approximately 3 weeks before blossoming. The whole pot, soil surface included, was covered with wax paper before inoculation of the seedlings. Three days after inoculation the paper was removed and the pots were again covered with fresh wax paper which was not removed for the duration of the experiments. The pots were watered from saucers to further minimize the possibility of contamination. Six plants were inoculated with each of the isolates 4, 10, and 32. Prior to inoculation, the leaves of 3 replicates were punctured to simulate flea-beetle injury. Control plants were sprayed with distilled water by means of an atomizer.

All plants were allowed to develop fruits under average greenhouse conditions. No water was applied to the foliage or pot surface at any time. About 3 months later, when the fruits began to mature, the plants were subjected to high humidity for 24 hours to enhance sporulation of the fungus on the infected foliage. Microscopic examination of the foliage showed that abundant sporulation had occurred. The foliage was then so arranged that it touched and overlaid the fruits in a manner similar to that found in the field. In the first experiment, foliage and fruits were allowed to touch the greenhouse bench which had been sterilized previously and covered with cellulose-acetate window cloth. In the second and third experiments, plants were placed on inverted pots to prevent the foliage and fruits from making contact with any water that might accumulate on the bench (Fig. 2, A). All plants were subjected to high humidity for 48 hours to afford optimum conditions for infection of the fruits by foliage inoculum. In all 3 experiments, anthracnose data were taken on fruits which ripened during the 3 weeks that followed the 48-hour humidity period. At the end of that period all plants still were bearing green fruits. The plants were restaked and no water was applied to the foliage or soil except by way of the saucers. The fruits were examined for anthracnose as they matured.

The summary of the results of all 3 experiments provides conclusive evidence that fungi which cause tomato anthracnose may be transmitted readily from infected foliage to the fruit (Table 3). Not only ripe fruits, but green fruits as well became infected in this manner and the latter developed typical symptoms when ripe (Fig. 2, B). None of the fruits from the control plants developed anthracnose. The marker isolate 32 was reisolated from both leaves and fruits

TABLE 3.—*Transmission of tomato anthracnose-causing fungi from infected foliage of greenhouse plants inoculated in the prefloral stage with isolates of* C. phomoides *and* G. cingulata[a]

Isolate number	Treatment of leaves at prefloral inoculation	Number of red fruits[b]		Number of green fruits[c]	
		Total	Infected	Total	Infected
4	punctured	17	14	32	14
4	not punctured	17	12	31	16
10	punctured	5	3	19	7
10	not punctured	3	3	32	16
32	punctured	13	10	27	8
32	not punctured	19	13	11	6
Control	punctured	18	0	36	0
Control	not punctured	18	0	28	0

[a] Figures represent the total number of fruits in 3 experiments. All 3 experiments gave consistent results.

[b] The number of fruits which ripened during the 3-week period following the 48-hour high humidity incubation period.

[c] The number of fruits which were still green at the end of the 3-week incubation, but which ripened later. Infection was determined after they had ripened.

on plants whose foliage was earlier inoculated with that isolate. Isolates 4 and 10 were similarly reisolated. Leaf punctures made at the time of prefloral inoculation did not influence the amount of fruit infection. It should be pointed out, however, that a number of the lower leaves of all plants were senescent or dead at the time they were placed in contact with the fruit. Such leaves supported a considerable amount of inoculum. A similar situation occurs in the field, where it has been repeatedly observed that infected fruits, frequently 6 in. or more above the soil, are overlaid with dead or senescent tissue that contains an abundance of spores (Fig. 3). Furthermore, such infected fruit often had lesions on the upper half of the fruit instead of on the lower half, as might be expected if the inoculum had come directly from rain-spattered soil particles.

The greenhouse results were confirmed in a field experiment conducted during the summer of 1954. Several flats of tomato plants at the transplanting stage (3-4 leaves fully expanded) were used. The plants were inoculated with isolate 32 and incubated for 48 hours in the greenhouse. Shortly thereafter, these plants and comparable noninoculated ones were planted in randomized blocks in the field. Each ripe fruit was examined and the amount of anthracnose recorded. In addition, at least 1 lesion from every infected fruit was plated out and cultured. The total percentage of fruits with anthracnose from the plants inoculated with isolate 32 before transplanting was 26.6, of which 1.1 per cent yielded cultures identical to isolate 32. Twenty-nine per cent of the fruit from noninoculated plants were infected but none of the reisolates resembled isolate 32.

Histology of foliage infection.—Stained sections prepared from inoculated leaves showed that both isolate 4 of *C. phomoides* and isolate 32 of *G. cingulata* penetrated the tomato leaf epidermis in less than 24 hours at 70-75°F. In the 24-hour section, a thin colorless penetration hypha was apparent in the epidermal cell. Germ tubes lying on the epidermis formed a number of appressoria along the line of growth. Most appressoria were located in the depression between 2 adjacent epidermal cells, although penetration in the middle of a cell was common. The contents of some cells carrying appressoria on their surface appeared granulated or deeply stained. Penetration occurred through either surface of the leaf and also through

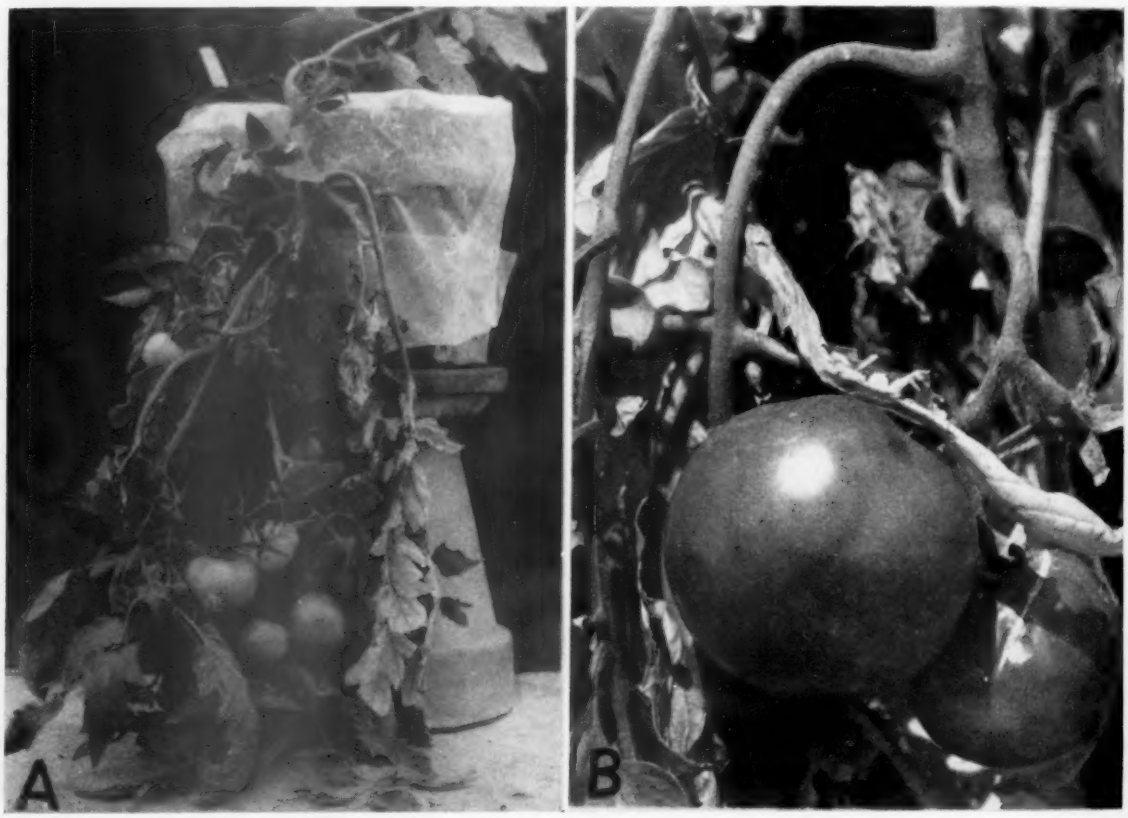

FIG. 2. A) Placement of potted tomato plant in studies on the transmission of anthacnose-causing fungi from leaves on which the fungus was sporulating to the fruit. B) Gem tomato fruits with anthracnose lesions resulting from infection by spores from infected foliage.

epidermal hairs. Advancement of a penetration hypha 48 hours after inoculation is shown in Figure 4, A. In the 72-hour section (Fig. 4, B), the hyphae in the epidermal cells appeared thickened and branched. They stained light purple with thionin. In more advanced stages, the fungus had permeated the leaf tissue, extended from one epidermal cell into another and into the mesophyll, and branched abundantly in the large palisade cells and spongy parenchyma. The fungus also grew inside and between the vascular bundles. The chloroplasts, which normally line the border of the palisade cells, appeared aggregated. In the 6-day sections, abundant acervuli were found on both leaf surfaces (Fig. 4, C).

Discussion.—The results presented in this paper indicate that the fungi which cause anthracnose of tomato fruit are capable of infecting root, stem, and foliage tissue, and that the mode of infection and subsequent fungal development, especially in leaves, is quite similar to that observed by Fulton (3) in green and ripe fruit. Furthermore, fungi that develop on foliage may constitute just as important a source of fruit inoculum as those from overwintered soil debris.

Root infection appeared to be of minor importance insofar as it might influence the development of fruit rot, but it may function in the overwintering of the fungus in tomato or other hosts. The fruit-rotting isolates used in these studies varied in their ability to infect and penetrate root tissue, but none progressed outward through the tissue to sporulate in the manner described for *C. atramentarium* (1, 2, 5, 6). None produced the aboveground effects attributed to that organism, although isolate 74 did produce similar root lesions. Whether a fruit-rotting Colletotrichum exists that functions like *C. atramentarium* remains to be determined. The pathogenicity of the latter organism on fruit has not been demonstrated except on punctured fruits (1), nor has it been observed in nature, possibly because most of the work was done on staked glasshouse crops where fruit anthracnose is not likely to be a problem.

The results obtained from the studies on foliage infection present a different aspect of the previously reported pathogenesis of tomato anthracnose. The infection of tomato foliage experimentally induced by others (4, 8) was confirmed. In addition, it was found that leaves of varying ages and locations on field-grown plants harbored numerous spores capable of producing fruit anthracnose. In some instances spore-bearing leaves were found on plants before fruit production had occurred. Sporulation was most abundant on the lower leaves that were in contact with the soil. That is tenable since such leaves are in closer contact with the primary inoculum from soil debris than those higher up on the plant. The fungus can also grow and sporulate more abundantly in the lower portion

Fig. 3.—Anthracnose lesions that developed naturally on the upper surface of fruit of field-grown plants. The dead leaves draped over the fruit contained an abundance of spores. No lesions were found on the soil-surface half of the fruit in the above cluster.

of the plant, because the leaves are more senescent and the conditions of humidity are more favorable. Furthermore, the lower leaves provide a better haven for the growth and sporulation of anthracnose-causing fungi because such leaves are either injured or killed earlier by foliage diseases than are the upper leaves. Histological studies showed that the fungi were able to penetrate the leaf epidermis in less than 24 hours but remained more or less latent until the leaf became physiologically more mature. This situation parallels the findings of Fulton (3) for fruit infection, except that the time interval between infection and sporulation in leaves was shorter than that between infection in green fruit and subsequent development in ripe fruit. It was also found that leaf injuries caused by flea beetles and Alternaria lesions provided loci for heavy development and sporulation of the anthracnose fungi. The role of foliage inoculum in the development of fruit rot was demonstrated in controlled greenhouse and field tests and was further substantiated by field observations. This conclusion is not in agreement with that of Kendrick and Walker (4), who minimized the importance of foliage inoculum because they were unable to recover an albino isolate from fruit on plants whose foliage was earlier inoculated with that culture. The discrepancy can be explained by the fact that they were unable to establish the pathogenicity of the albino strain except on punctured fruit, whereas in these investigations the pathogenicity of the marker strain, isolate 32, was proved conclusively on uninjured fruit. In this connection it should be noted that 2 of the isolates used in the present studies, numbers 1 and 5, were albino, and their pathogenicity was not demonstrated except on punctured fruit. All pigmented isolates, however, were highly infectious on uninjured fruit.

In the opinion of the writers, foliage inoculum as described in these studies offers a more plausible basis for explaining many of the reported observations on the incidence of field anthracnose than does any previously suggested inoculum source. For instance, the heavy development of anthracnose in early and

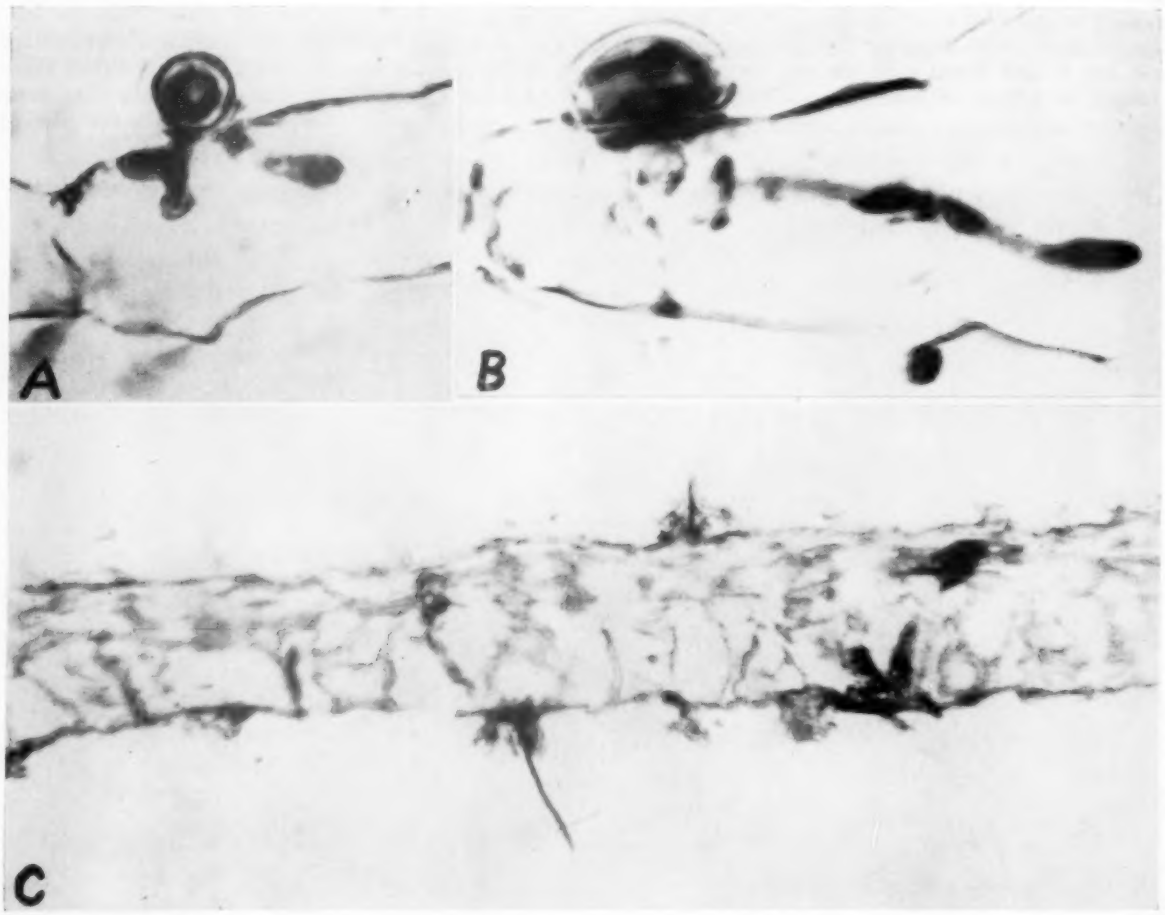

FIG. 4.—Penetration of tomato leaves, variety Gem, by *C. phomoides*, isolate 4. A) Penetration beneath appressorium and growth of the tube into epidermal cell, 48 hours after inoculation. B) Hyphal growth into epidermal cell, 72 hours after inoculation. 2300 ×. C) Development of acervuli on both leaf surfaces, 6 days after inoculation. 300 ×.

determinate varieties and in those subject to early defoliation, as reported by Wilson and Runnels (7), could result just as logically from heavy foliage-inoculum that developed in the injured or senescent leaf tissue as from primary soil-inoculum. Foliage inoculum could also explain the unusually heavy development of anthracnose that can occur during periods of heavy and prolonged dews in the absence of rainfall. Further substantiation is afforded by field observations wherein anthracnose lesions often are found on the upper surface of fruits which are overlaid with spore-bearing senescent or dead leaves. The fact that lesions often are found on the soil-surface side of the fruit does not rule out the importance of foliage inoculum because fruits frequently are found resting on dead or senescent leaves. Furthermore, any rain or dewdrops would naturally follow the curvature of the fruit and hang at the bottom. Also, the fruit and foliage areas closest to the soil are favored by a longer and higher humidity period. The low incidence of anthracnose on staked tomatoes is frequently ascribed to the fact that the tomatoes are not in contact with the soil. It could just as conceivably be due to the fact that the fruits are generally above the older more mature leaves and thus not in contact with foliage inoculum, although the more favorable air drainage is probably a better reason than either of the aforementioned. Wilson and Runnels (7) pointed out that detached fruits from some varieties that show some field tolerance to anthracnose are more susceptible when artificially inoculated than are similarly detached fruits from some varieties that show a high degree of field susceptibility. A study of the varietal relationship between foliage inoculum potential and fruit susceptibility in the field might prove of value. Foliage inoculum might also explain why early sprays, applied before most of the fruits are formed, are so important in the successful field control of the fruit disease, for it was pointed out in these studies that tomato plants set in the field showed foliage infection before floral development. It is not the intention of the writers to minimize the importance of primary soil inoculum in the development of tomato fruit anthracnose, but to suggest that it might also provide primary inoculum for tomato foliage and cause infections which in turn could induce secondary fruit infections of possibly greater importance.

Department of Plant Pathology
New York Agricultural Experiment Station
Geneva, New York

LITERATURE CITED

1. Bewley, W. F., and J. Shearn. 1924. A root disease of the tomato caused by Colletotrichum tabificum (Hallier pro parte) Pethybridge. Ann. Appl. Biol. **11**: 224-251.
2. Colquhoun, T. T. 1941. Black dot root rot of tomatoes. So. Austral. Dept. Agr. Jour. **44**:572-575.
3. Fulton, J. P. 1948. Infection of tomato fruits by Colletotrichum phomoides. Phytopathology **38**:235-246.
4. Kendrick, J. B., and J. C. Walker. 1948. Anthracnose of tomato. Phytopathology **38**:247-260.
5. McKay, R. 1942. Tomato root rot, Colletotrichum atramentarium (Berk. & Br.) Taubenh. Eire Dept. Agr. Jour. **39**:272-276.
6. Williams, P. H., and Marion Ebben. 1951. Brown root rot of the tomato. [Cheshunt] Exp. and Res. Sta. Ann. Rpt., 1950, p. 23-26.
7. Wilson, J. D., and H. A. Runnels. Tomato anthracnose. Comparative susceptibility of varieties and their response to spraying. Ohio Agr. Exp. Sta. Res. Bul. 685, 22 p.
8. Younkin, S. B., and A. W. Dimock. Foliage infections of Lycopersicon esculentum by Colletotrichum phomoides. Phytopathology **34**:976-977.

REPORT AND ABSTRACTS OF THE 1955 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Southern Division of the American Phytopathological Society met jointly with the Association of Southern Agricultural Workers at its 52nd annual convention at Louisville, Kentucky, on February 7, 8, and 9. Thirty-seven papers were presented and more than 100 pathologists attended. The symposium on Forage Crop Diseases and Glassland Farming, held in conjunction with the Crops Section of the American Society of Agronomy, was attended by more than 200 people.

The following officers were elected at the business meeting on February 7: *President*, T. W. Graham; *Vice-President*, J. A. Lyle; and *Councilor*, J. L. Allison.

G. B. Lucas, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Morphological and physiological comparison of certain isolates of Phytophthora parasitica var. nicotianae. Apple, J. Lawrence. Differences in colony morphology, growth rate, sexuality, and pH optimum have been found among isolates of the tobacco black shank fungus (*P. parasitica* var. *nicotianae*) from different geographic locations. On tobacco-extract and V-8-juice agars, isolates from burley-tobacco areas of North Carolina, Tennessee, and Kentucky produced smooth rapidly expanding colonies with definite margins and aerial mycelium. Isolates from flue-cured tobacco areas of North Carolina produced irregular slow-growing colonies of submerged abruscoid mycelium. The optimum pH range for isolates from burley tobacco was more restricted and slightly lower than for isolates from flue-cured tobacco. Homothallic and 2 heterothallic compatibility types have been found. Among the heterothallic types, only 1, tentatively considered antheridial, was found in the flue-cured area of North Carolina and only 1, presumably oogonial, was found among isolates from burley tobacco. Burley × flue-cured matings usually produced oospores but never abundantly, regardless of the medium used. When an isolate of *P. capsici* from squash was mated with isolates from flue-cured tobacco, oospores formed abundantly, but when such isolates were mated with isolates from burley tobacco, no oospores were formed. Isolates of the black shank fungus from other sources were sexually similar to isolates from North Carolina flue-cured tobacco.

Pathogenic variation among single zoospore isolates of Phytophthora parasitica var. nicotianae. Apple, J. Lawrence. In inoculation experiments, isolates of the black shank fungus (*P. parasitica* var. *nicotianae*) have been found to vary from a low level of pathogenicity on a susceptible tobacco variety (Bottom's Special) to a high level of pathogenicity on a moderately resistant variety (Dixie Bright 101). A 4-year-old culture was found to be weakly pathogenic although it was highly pathogenic when isolated. Fifteen single-zoospore isolates from this culture were studied. Some cultural variability with respect to growth rate and colony morphology was apparent among single-zoospore isolates but the most significant differences were in pathogenicity. They varied from nonpathogenic to highly pathogenic, the latter extreme greatly exceeding the pathogenicity of the parent culture. This variation probably has arisen through mutation in culture. These observations are significant in that they demonstrate some of the variation potential of the black shank fungus.

Host relationships of the wilt Fusaria from spinach and beet. Armstrong, J. K., and G. M. Armstrong. Single spore lines of the spinach wilt Fusarium caused severe wilting of Aragon, Nobel, and Bloomsdale Savoy varieties of spinach. They also caused wilting of sugar and garden beets and some varieties of chard, although their pathogenicity for these crops was considerably less than that of the beet wilt fusarium. Single spore lines of the beet wilt Fusarium caused severe wilting of sugar and garden beets, less severe wilting of chards and mangel, and no wilting of the 3 varieties of spinach. Kochia was not affected by either Fusarium. The spinach and beet wilt Fusaria produced no external symptoms of wilt on susceptible varieties of 25 species of plants, other than the Chenopodiaceae when grown in 2-gal. pots of sand in the greenhouse. Under similar conditions, 27 different wilt Fusaria of the section Elegans, exclusive of the spinach and beet Fusaria, produced no external symptoms of wilt on a susceptible variety of sugar beet and of spinach. The results indicate that the beet wilt Fusarium should be designated as a race of the spinach Fusarium, since beet and chart are the common hosts.

The sumac wilt fungus a race of the aster wilt Fusarium. Armstrong, J. K., and G. M. Armstrong. Small plants of sumac (*Rhus typhina* L.) and aster growing in 2-gal. pots of sand are nearly all killed in less than 2 months after inoculation with single spore lines of the sumac wilt fungus; however, sumac is unaffected by the aster wilt Fusarium which is highly pathogenic on susceptible varieties of aster. Plants of 29 different species and varieties, other than aster and sumac, showed no external symptoms of wilt after inoculation with either the sumac or the aster wilt Fusarium. Twenty-seven different wilt Fusaria of the section Elegans, excluding the aster and sumac wilt Fusaria, produced no external symptoms of wilt on sumac and a susceptible variety of aster. Since aster is a common host, the sumac wilt Fusarium should be designated as a race of the aster wilt Fusarium.

Pathogenicity of plant-parasitic nematodes in the absence of associated microorganisms. Cairns, Eldon J. Experimental work with a species of *Ditylenchus* parasitizing mushrooms provided opportunity to determine if a plant-parasitic nongall-forming type of nematode could cause extensive injury to its host in the absence of associated microorganisms. Inoculation of mushroom cultures with disinfested nematodes resulted in diseased colonies within 4 to 7 days. Mushroom cultures inoculated with contaminants only showed injury restricted to the edges of cuts made in the mycelia to simulate the wounding action of nematode feeding. Cultures artificially wounded and inoculated with fragmented surface-disinfested nematodes showed no extension of injury away from the wounds, nor was there evidence that disease-producing agents were contained within the nematodes. Cultures artificially wounded and "inoculated" with sterile water used in the nematode disinfesting procedures showed no extension of injury away from the wounds. The experiment demonstrated that living *Ditylenchus* sp. could, in the absence of other organisms, cause injury and eventual destruction of its host, *Agaricus campestris* L. Microorganisms associated with the nematodes on diseased mushrooms were not able to

cause extensive damage without the presence of the nematodes even though aided by artificial wounding of the mycelia.

Root knot nematode resistance of soybeans. CRITTENDEN, H. W. Fifty varieties of soybeans have been tested in Delaware for resistance to *Meloidogyne incognita* var. *acrita*. These varieties include representatives from all the maturity groups from O to VIII. Varieties in group O are suitable for the northern United States and those in group VIII are grown in the Gulf-Coast region. The following 10 varieties have shown high resistance: Laredo (Group VI), Mukden (II), Anderson (IV), Monroe (I), Blackhawk (I), Peking (IV), Mendota (II), Haberlandt (V), Habaro (I), and Mandarin 507 (I). The 5 varieties having the highest resistance to *M. incognita* var. *acrita* have been tested also against *Meloidogyne hapla*—all 5 (Laredo, Mukden, Anderson, Monroe, and Blackhawk) were susceptible. These findings emphasize that: 1) the growing of a crop resistant to *M. incognita* var. *acrita* may selectively increase populations of *M. hapla;* and 2) until combined resistance is obtained, it appears essential to know the identity of the root knot nematode species in any area where rotations are used for control.

Organic mercury dips for the control of nematodes in roots of living plants. FELDMESSER, JULIUS, AND WILLIAM A. FEDER. Two organic mercurials, Aaventa and Aabulba, show promise of controlling the golden nematode (*Heterodera rostochiensis*) adherent to roots and the root-lesion nematodes (*Pratylenchus* spp.) and the root-knot nematodes (*Meloidognye* spp.) within the roots of living plants. Two hundred and eighty dip-tests showed that exposures to 2 per cent for 10 minutes, to 1 per cent for 1 hour, to ½ per cent for 3 hours, or to ¼ per cent for 6 hours of either chemical were lethal to larvae within golden nematode cysts. Bare-rooted ornamentals infected with *Pratylenchus* spp. and *Meloidogyne* spp. were freed of their viable nematodes after 1-hour dip treatment with Aaventa. Periodic observations indicated that nematodes probably are killed within 48 hours after treatment. Aaventa proved to be less phytotoxic than Aabulba. However, both were equally nematocidal. A number of commercially important ornamental plants of genera such as *Philodendron, Pothos, Peperomia,* and *Sansevieria* have been successfully treated.

Peach brown rot and scab control in South Carolina. FOSTER, H. H. During the past 2 years, 15 different fungicides or fungicide combinations have been tested, under orchard conditions, for the control of peach brown rot and scab. Wettable sulfur (6 lb./100 gal.) and captan (50 per cent wettable, 2 lb./100 gal.) were the most effective fungicides. Greater advantage in disease control was not obtained from the use of double (2 × sprays) the above mentioned rates with the gallonage delivered reduced to approximately ½ the amount used for the dilute (1 ×) sprays. In 1954, following a 4-day holding test with orchard-sprayed peaches from 2 comparable blocks (Triogem variety), 1.3 per cent of the captan-sprayed fruits had brown rot symptoms as compared with 17.2 per cent of sulfur-sprayed peaches. In addition, captan-sprayed peaches were compared with sulfur-sprayed fruits after 10 and 14 days storage at room temperature. The number of healthy peaches free of brown rot and Rhizopus lesions was consistently greater for captan-sprayed fruits. At 2 locations in 1954, captan was somewhat less effective than wettable sulfur in controlling the scab fungus. During periods of relatively high temperatures, applications of wettable sulfur have increased a sunscald and spray-burn injury with peaches. Captan-sprayed peaches usually had an excellent finish.

Pathogenicity of Rotylenchus brachyurus on tobacco and of Criconemoides sp. on tobacco and peanuts. GRAHAM, T. W. One of the spiral nematodes, *Rotylenchus brachyurus,* was established in greenhouse soil cultures for pathogenicity trials on Golden Cure tobacco. Nematodes increased equally well on sweet potatoes, tobacco, or *Crotalaria striata.* Populations of about 4000 to 5000 per gal. of soil were necessary to cause stunting of tobacco plants grown in infested soil where root weight, top weight and height measurements were respectively 57, 43, and 40 per cent less than those of plants grown in nematode-free soil. In field pathogenicity tests on Hicks tobacco, plant height was significantly less in infested plots than in control plots but green weight of harvested leaves, of stalks, or of roots did not differ. Root injury was slight to moderate in both greenhouse and field trials. Although root lesions were present, the generalized root decay observed following meadow nematode invasion was not found. The ring nematode, *Criconemoides sp.,* was studied similarly in the greenhouse on tobacco and on Spanish peanuts. Both crops when grown in infested soil were stunted in height and in green weight of tops and roots. Peanuts infected with ring nematodes showed considerable root decay. Symptoms on tobacco roots infected with ring nematodes were similar to those infected with spiral nematodes and therefore showed very little root decay.

Resistance to Fusarium wilt in Brown Sugar Crowder cowpeas. HARE, W. W. The Brown Sugar Crowder cowpea is a distinctive variety popular for canning, shipping, and home gardens. In tests for Fusarium wilt resistance, it was 1 of the few commercial varieties with no resistance or tolerance to the 3 known races of *Fusarium oxysporum* f. *tracheiphilum.* A new strain of the Brown Sugar Crowder type, designated S-1, originated from a single surviving plant of the parent type in a wilt plot in 1949 and has high resistance to race 1, tolerance to race 2, and a lesser tolerance to race 3 of the wilt organism. Tolerance is defined as a slow rate of killing in the greenhouse and low percentages of wilt under field conditions. In a greenhouse inoculation test with pure cultures, all Brown Sugar Crowder plants were killed, and 0, 46, and 71 per cent of S-1 plants were killed by races 1, 2, and 3, respectively. In a field plot in 1954 where all 3 races were present, the average kill by wilt in 4 replications was 82 per cent for Brown Sugar Crowder and 13 per cent for S-1 at the end of the harvest season.

Resistance to Cercospora in pepper. HARE, W. W. Leaf blight incited by *Cercospora capsici* has caused severe losses in Mississippi by defoliation of pimiento and pickle peppers grown for ripe fruit. In 1951, pepper varieties and strains growing in a nonreplicated field plot showed apparent differences to natural infection by *C. capsici.* Infection was rated zero, trace, slight, moderate, and severe. The following winter in the greenhouse, 4 replications of 42 varieties rated slight or less, or uncertain, were inoculated with the fungus. Young plants grown in flats were used. Results varied but varietal differences were substantiated. Tests were repeated in 1952-53 with 22 selected varieties. In 1953-54 the 4 most resistant varieties were compared to susceptible California Wonder as young plants in flats and also as mature fruiting plants in 8-in. pots. On young resistant plants in flats, scattered small leaf-lesions developed slowly with no defoliation. California Wonder had numerous large leaf-lesions resulting in severe defoliation. Mature California Wonder plants were susceptible while the 4 resistant varieties showed only an occasional slight marginal leaf-scorch. These resistant varieties were small-fruited hot peppers from Brazil and Puerto Rico.

Damping-off of tomato transplants. HARRISON, A. L. Losses up to 80 per cent from damping-off of field-set tomato plants have been experienced by tomato growers in parts of the South during the hot summer months of July and August. Rarely has this phase of damping-off caused serious losses during the cool spring weather. Most of the affected plants die within 3 to 5 days after transplanting, although some plants may damp-off within 24 hours. Succulent plants are more susceptible than properly hardened plants. *Pythium* sp. has usually been isolated from damped-off plants although *Rhizoctonia solani* has occasionally been isolated. Randomized plantings in the greenhouse in soil artificially infested with oat cultures of either the *Pythium* sp. or *Rhizoctonia solani,* and observational tests in the

field have demonstrated that losses from damping-off of tomato transplants can be reduced by adding Puratized C12-2525 (anilinocadmium lactate; phenylmercury derivative of 2-mercaptobenzothiazole), ferbam, Copper Compound A (basic copper chloride), Crag Potato Fungicide (copper zinc chromate, $2.5CuO \cdot 0.6CdO \cdot ZnO \cdot 10CaO \cdot CrO_3$), Vancide 51ZW (zinc dimethyldithiocarbamate; zinc 2-mercaptobenzothiazole), ziram, or zineb to the transplant water. Some fungicides controlled damping-off but were too phytotoxic at effective concentrations. At a low inoculum potential, some treatments gave 95–100 per cent control, while at a high inoculum potential, the best treatments frequently gave less than 50 per cent control.

Rod-shaped particles associated with soil-borne wheat mosaic. HEBERT, T. T., AND N. T. COLEMAN. Electron microscope studies revealed the presence of rod-shaped particles in juice from wheat plants infected with the soil-borne mosaic virus, *Marmor tritici*. Such particles were not found in juice from healthy wheat plants. Coarse plant debris was first removed from the juice by centrifugation at 10,000 *g* for 10 minutes. The supernatant was then centrifuged at 25,000 *g* for 1 hour and the pellet formed was suspended in distilled water. Electron-microscope mounts were made from this suspension. The particles were approximately 28 mμ in diameter and varied in length between 400 and 700 mμ. At pH 7, the particles were precipitated from centrifuged suspensions by 25 per cent ammonium sulfate but not by 15 per cent ammonium sulfate. Partially purified preparations obtained by centrifugation followed by fractional precipitation with ammonium sulfate showed stream double refraction and were highly infectious. When young wheat plants were inoculated by leaf rubbing with 1 partially purified preparation at dilutions of 1:20, 1:400, and 1:8000, and with undiluted juice from infected plants, the percentage of plants infected was 100, 97, 25, and 89, respectively.

Burley, flue-cured, and cigar-wrapper tobacco varieties compared for resistance to black shank and Fusarium wilt. HEGGESTAD, H. E. Two recently released tobacco varieties, Burely 11A and Burley 11B, were found to be more resistant to black shank (caused by *Phytophthora parasitica* var. *nicotianae*) than 6 flue-cured resistant varieties, although not as resistant as the cigar-wrapper varieties Dixie Shade and R. G. In 1954, plantings were made on 2 East Tennessee fields infested with the black shank organism. On Sept. 3, Dixie Shade and R. G. averaged 95 and 89 per cent survival, respectively, compared to 86 and 81 per cent for Burley 11B and Burley 11A, respectively. Flue-cured varieties Dixie Bright 101 and 102 and Vesta 64, 33, 5, and 47 ranged from 33 to 13 per cent survival. Burley 11B and Burley 11A are significantly more resistant to Fusarium wilt (caused by *Fusarium oxysporium* var. *nicotianae*) than the wilt-resistant commercial variety Kentucky 35. In 1953, plants were inoculated with fungus isolates from the burley area; in 1954, the inoculum included several isolates from both burley and flue-cured areas. Plant survival in 2 years' tests averaged 62, 42, and 16 per cent, respectively, for Burley 11B, Burley 11A, and Kentucky 35. In 1954, wilt-resistance was also determined for the flue-cured varieties Dixie Bright 101 and 102, Vesta 33 and 64, and Va. Gold, and the cigar-wrapper varieties Dixie Shade and R. G. All varieties had wilt resistance equal to or better than that of Burley 11A.

Effect of soil insecticide treatments on alfalfa stem nematode. HENDERSON, R. G., AND A. S. WILLIAMS. In a test conducted in the spring of 1954, soil infested with the alfalfa stem nematode (*Ditylenchus dipsaci*) was treated with aldrin and parathion dust at the rate of 2 lb. and 5 lb. of the active chemical, respectively, per acre. The insecticides were applied broadcast and then disked into the soil. The plots were seeded immediately after treatment (April 14) to alfalfa in the customary manner. By May 12, a striking contrast was evident between the treated and untreated plots. The seedlings on the treated plots appeared healthy and, considering the deficiency of soil moisture, were growing vigorously. The stand was irregular because of dry weather; but, in general, it was good on the aldrin-treated plots and medium on the parathion-treated plots. On the untreated plots the stand consisted of scattered plants which were yellow and dwarfed. The stems at the ground line were enlarged and laboratory examinations revealed the presence of nematodes of the *D. dipsaci* type in the tissue. Many of the diseased plants made a remarkable recovery in hot weather. During the hot dry summer, the nematodes were inactive in the plants, but following a fall rain they could be found in some of the new shoots.

Evidence of seed transmission of the cucumber anthracnose pathogen. HORN, N. L., AND W. F. WILSON. Transmission of *Colletotrichum lagenarium* through the seed of cucumbers has been suspected for years, but no experimental evidence demonstrating this condition has been recorded in the literature. In order to determine whether or not the organism is seed transmitted, approximately 50,000 seedlings were grown from commercial seed in steamed soil in the greenhouse and examined for anthracnose spots. Six were found to be infected with the organism. Although a low percentage of transmission occurred, it initiated field infection under favorable weather conditions. The pattern of infection in the field when the disease first appeared suggested seed transmission. Plants grown from disease-free seed did not become infected when grown in the field. Disease was equally as severe on plants grown in soils never before planted to cucumbers as on plants grown in fields where there was a disease the previous year. Seedlings were severely diseased when grown outdoors in artificially infested soil. However, no plants were diseased when grown in the same soil 10 months later. Soil was collected where the disease first appeared in the field and placed outdoors in flats. Seedlings grown in this soil did not become infected.

Variation in pathogenicity of isolates of Pseudomonas solanacearum on different hosts. KELMAN, ARTHUR, AND L. H. PERSON. An explanation was sought for low incidence of Granville wilt on tobacco in areas in Georgia where the disease is prevalent on tomato. In greenhouse tests for pathogenicity 16 isolates of *Pseudomonas solanamearum*, obtained from tomato plants collected in 10 Georgia counties, were compared with 2 tobacco and 2 tomato isolates from North Carolina. A stem-puncture technique was used to inoculate tomato, tobacco, eggplant, Irish potato, and peanut plants with suspensions of the organism. All isolates were highly and equally pathogenic to tomato, Irish potato, and eggplant, and killed the plants in 7–12 days. Differences in virulence of isolates appeared on tobacco, and were more evident on the moderately resistant variety, Dixie Bright 101, than on the susceptible variety, Bottom's Special. On Dixie Bright 101, 2 Georgia isolates were highly pathogenic and 14 were moderately to weakly pathogenic; 3 North Carolina isolates were highly pathogenic and 1 from tomato was moderately pathogenic. Some isolates weakly pathogenic on tobacco were highly pathogenic on peanut; the reverse was true for some isolates. Certain isolates were equally pathogenic on both hosts. These preliminary data show that there may be different pathogenic strains of *P. solanacearum*.

Chemotherapy of the peach rosette virus with antibiotics. KENKNIGHT, GLENN. Certain Lovell peach seedlings, inoculated with peach rosette virus by bark-patch grafts in August 1953, expressed symptoms in October. Partially to fully rosetted trees were lifted in March 1954, when they were breaking dormancy. Of 22 untreated controls, 8 developed further rosetted growth and 14 died without appreciable growth. Roots and lower stems of 38 affected trees were immersed 4 hours in 10 qt. of various antibiotic solutions. Of 19 treated with cycline compounds, 11 made various degrees of recovery. Six appeared normal in October 1954: 2 receiving achromycin (tetracycline complex) at 5 ppm active ingredient; 2, achromycin at 25 ppm; 1, agrimycin (1 part terramycin to 10 streptomycin) at 5

ppm; and 1, terramycin (oxytetracycline) at 25 ppm. On all 6 trees, initially rosetted limbs died early in the season. Five trees showed temporary recovery: 2 receiving aureomycin hydrochloride (chlortetracycline) at 25 ppm; 1, agrimycin at 5 ppm; and 2, agrimycin at 25 ppm. Initially rosetted growth died on 3 of these 5 trees. No recovery was obtained with 25 ppm Acti-dione (cycloheximide), endomycin, neomycin, or streptomycin. Five 2-year-old fully rosetted trees were injected in May 1955, each with 50 mg achromycin in 2 ml water in a bore-hole in the trunk. Two trees made temporary recovery.

Role of black peach aphid in spread of crown gall. KEN KNIGHT, GLENN, AND JULIAN JONES. In a screenhouse erected at Fort Valley, Ga., every peach tree of the original 800 planted developed crown gall. All trees had been infested with black peach aphids. Crown gall was uncommon out-of-doors and so were black peach aphids. In 3 subsequently erected screenhouses, the incidence of galls seemed closely related to local infestations of the aphid. On March 8, 1952, black peach aphids were fed overnight on galled peach roots, then transferred and allowed to remain for 4 hours on sprouted Lovell peach seed. The seed was then planted in sterilized orchard soil in a greenhouse. The experiment was repeated in 1953 and 1954. For the 3 years, the numbers of trees developing galls after being infested with aphids from galled peach roots were 9 of 15, 3 of 19, and 9 of 30, respectviely. Among control trees planted in sterilized soil, the numbers of galled trees were 0 of 10, 0 of 16, and 1 of 13, respectively. Among control trees planted in nonsterilized soil, the numbers were 0 of 16, 2 of 19, and 0 of 33, respectively. There appears to be an association between activities of the black peach aphid and development of crown gall on peach trees.

The lance nematode, Hoplolaimus coronatus, a parasite of cotton. KRUSBERG, LORIN R., AND J. N. SASSER. Soil and root samples taken from a cotton field showing stunted and irregular plant growth were found to contain a high population of the lance nematode, *Hoplolaimus coronatus* Cobb. Ten soil samples taken from around the roots of stunted cotton plants averaged 1495 nematodes per pint. Ten soil samples taken from around the roots of apparently healthy nearby cotton plants averaged 125 nematodes per pint. All stages of the nematodes were found in the roots of stunted plants. Other parasitic forms (*Helicotylenchus* sp., *Pratylenchus* sp., and *Meloidogyne* sp.) were also present but they were no more numerous in samples from areas containing stunted plants than in those from areas where plants were healthy. Feeding habits of the lance nematode were observed in the laboratory by placing nematodes around the roots of cotton seedlings growing in sand in test tubes. It was found to feed externally, partially embedded or completely embedded in the root tissues. A yellowish-brown discoloration of the root tissue occurred at points of nematode penetration and feeding. When 100 lance nematodes were added directly beneath a single cotton seed in each of 10 test tubes containing sand, 7 plants emerged and those were markedly stunted. In the controls, all 10 plants emerged and made good growth. This nematode has not previously been reported as a parasite of cotton.

Relationship of nematodes of the genera Meloidogyne, Tylenchorhynchus, Helicotylenchus, and Trichodorus to the development of Fusarium wilt in cotton. MARTIN, W. J., L. D. NEWSOM, AND JACK E. JONES. Studies were made of the development of wilt in cotton grown in steam-sterilized soil which was artificially infested with nematodes alone, with *Fusarium oxysporum* f. *vasinfectum* alone, and with a combination of nematodes and Fusarium. Pure populations of the nematodes *Meloidogyne incognita* var. *acrita*, *Tylenchorhynchus* sp., *Helicotylenchus* sp., and *Trichodorus* sp. were used. These nematodes were found to feed and reproduce abundantly on cotton varieties Deltapine 15 and Coker 100 WR. Of the 4 nematodes, only *M. incognita* var. *acrita* significantly increased the incidence of wilt in these 2 varieties of cotton.

High black shank resistance and tolerance to parasitic nematodes in flue-cured tobacco. MOORE, E. L., P. N. DROLSOM, AND E. E. CLAYTON. Over a 5-year period, 1949-1953, black-shank-resistant Dixie Bright 101 produced yields of cigarette tobacco comparable to those of the widely grown but susceptible flue-cured tobacco varieties in North Carolina. In black shank nurseries, from 1949 to 1951, less than 15 per cent of the Dixie Bright 101 plants were diseased, while over 98 per cent of the susceptible check plants were killed. In 1951, the first year large acreages of Dixie Bright 101 were planted, some fields showed 50-90 per cent losses from black shank. During the same and subsequent years, in some black-shank-free fields, Dixie Bright 101 was critically stunted; while intermixed plants of black shank susceptible Hicks and certain broad-leaved varieties were vigorous. Parasitic nematodes, including *Tylenchorhynchus claytoni*, *Meloidogyne* sp., and *Pratylenchus* sp., were found in all cases where Dixie Bright 101 was stunted. Dixie Bright 102, closely related to Dixie Bright 101 but with higher black shank resistance and lower yield, was crossed to susceptible varieties, Hicks and Bottom Special. The latter was further crossed in the F_2 to Dixie Bright 101. F_6 lines from the Hicks cross had higher resistance than, and F_5 lines from the Bottom Special cross the equivalent resistance of, Dixie Bright 102. The lines had tolerance to root knot and meadow nematodes. Yields and quality exceeded those of Dixie Bright 101 on disease-free soil.

Variable effects of nematocides on parasitic nematode populations in row-fumigated tobacco plots. NUSBAUM, C. J. In 1954, soil fumigation tests for nematode control were conducted on 16 tobacco farms in 12 counties in central and eastern North Carolina. Pretreatment nematode assays of soil samples from these plots showed that root knot nematodes (*Meloidogyne* spp.) and meadow nematodes (*Pratylenchus* spp.) were present at all locations; stunt nematodes (*Tylenchorynchus claytoni*), spiral nematodes (*Helicotylenchus* spp.) and dagger nematodes (*Xiphinema americanum*) were found at 13, 5, and 9 locations, respectively. Three nematocides were applied in the row with gravity flow equipment at the following rates per lineal ft.: Dowfume W-85 (1,2-dibromomethane) 0.65 ml; D-D mixture (1,3-dichloropropene; 1,2-dichloropropane), 3.26 ml; and PN-20 (20 per cent 1,2-dibromomethane in Phillips hydrocarbon mixture), 2.44 ml. Each treated and check plot was approximately 0.075 acre in size. Root and soil samples for nematode assay were collected from each plot in midseason and after harvest. All 3 treatments generally gave satisfactory root knot control, but D-D was superior to the others in 2 tests located on coarse sandy soils. Both formulations of 1,2-dibromomethane markedly reduced stunt-nematode populations and virtually eliminated spiral and dagger nematodes, but were much less effective than D-D mixture against meadow nematodes. However, D-D was less effective than the other treatments against stunt nematodes.

Comparison of Dowfume W-85 and D-D mixture applied as liquids and impregnated on vermiculite for nematode control. NUSBAUM, C. J., AND J. N. SASSER. Row applications of Dowfume W-85 (1,2-dibromoethane) and D-D mixture (1,3-dichloropropene; 1,2-dichloropropane) were made 2 weeks before planting tobacco. The fumigants were applied as liquids with a gravity-flow applicator and as solids, i.e. liquids impregnated on No. 3 grade vermiculite, with a fertilizer distributor. The fumigants were applied at 8 in. depth in 2-row plots replicated 3 times. Controls received no fumigant. Root-knot indices were taken on plants from each treatment at the end of the growing season. All treatments had significantly lower indices than the control, which had an index of 91.7. Indices for certain treatments at indicated rates per acre follow: Dowfume W-85, 2 gal. in 120 lb. of vermiculite—7.3; Dowfume W-85, 2 gal. in 60 lb. of vermiculite—16.7; Dowfume W-85, 2 gal., liquid—18.9; D-D, 10 gal. in 60 lb. of vermiculite—20.4; and D-D, 10 gal., liquid—33.7. In general, fumigants impregnated on vermiculite gave slightly better root knot control than comparable liquid applications, thus demonstrating the

practicability of this method of soil fumigation.

Studies on barley scald and its control in Tennessee. REED, H. E. Scald first appears in East Tennessee in mid-winter with greatest leaf-lesion development occurring in April. New lesions continue to develop on new foliage until harvest in June. Conidia are short-lived, and overseasoning is by mycelium in barley refuse and seed. In continually barley-seeded plots, scald first appeared on plants at the edges adjacent to plot borders where refuse from scald-diseased barley remaining from the preceding season was left exposed. Covering such refuse prevented the disease in barley in plot interiors. In field and laboratory studies, the fungus in the barley refuse failed to produce conidia or the disease the second season, indicating the value of a 2-year rotation. Occurrence of scald on barley seeded in areas previously scald-free indicated that the primary inoculum came from diseased seed or particles of refuse from diseased barley accompanying the seed, which remained uncovered, especially at ends of drill rows. Seed transmission of the fungus was verified later by controlled field experiments. In tests on scald-diseased barley seed and leaves, Panogen (cyano(methylmercuri)guanidine) and hot-water treatments completely suppressed sporulation of *Rhynchosporium secalis.*

Reduction of root-parasitic nematode populations in established bent grass turf by use of chemical drenches. TARJAN, A. C., AND P. C. CHEO. Substantial numbers of 6 species of root-parasitic nematodes were found in soil samples from a 6-year-old planting of Seaside creeping bent grass. The following chemicals were applied in aqueous formulations as soil drenches to 5 replicated 25-sq.-ft. plots at the rate of 50 gal. per 100 sq. ft.: Heptachlor (1,4,5,6,7,8,8-heptachloro-3a, 4,7,7a-tetrahydro-4,7-methanoindene) at 300 lb. per acre; N-244 (3-*p*-chlorophenyl-5-methylrhodanine) at 600 and 400 lb. per acre; V-C 1-13 (a neutral ester of phosphorothioic acid) at 500 and 250 lb. per acre; and R-I-26 (an unsaturated aliphatic compound) at 192 and 96 lb. per acre. Control plots received the acetone and Triton X-100 adjuvants without toxicants, since these were used in the emulsification of Heptachlor and N-244. Chemical injury developed in plots receiving N-244; the higher dosage killed the turf. Approximately 14 weeks after treatment, soil samples from each treated plot were examined both qualitatively and quantitatively for nematode content. Nematode populations in all treated plots were lower (difference highly significant statistically) than populations in the untreated plots. V-C 1-13 and Heptachlor were the most effective nematocides among those tested.

Some hosts of the pink root fungus (Pyrenochaeta terrestric) in Louisiana. TIMS, E. C. The onion pink root fungus is known to occur on onion, shallot, and garlic in Louisiana. The fungus has been isolated also from the roots of a number of other plants, but pathogenicity tests with such cultures have not been reported. During 1953 and 1954, the pink root fungus was isolated from roots of a number of weeds and grasses, as well as corn, soybean, and tomato growing in fields known to be infested. There was no pink discoloration or other typical lesion on the roots of these plants. Cultures obtained from the roots of corn, soybean, tomato, pig weed (*Amaranthus retroflexus*), crab grass (*Digitaria sanguinalis*), crow foot grass (*Eleusine indica*), and jungle rice (*Echinochloa colona*) were typical of those isolated from onion and shallot. Texas grano onion seedlings were inoculated with 1 or more cultures from each of these kinds of plants. In all the tests, typical pink root symptoms were observed, similar in every respect to those obtained with cultures from onion and shallot. These results indicate that a number of crop plants as well as some grasses and weeds may serve as hosts for the pink root fungus in the absence of the cultivated Alliums.

Some observations on the pathological histology of downy mildew of corn. WHITEHEAD, MARVIN D. Systemic infections which resulted in cell hypertrophy and hyperplasia were observed. In leaf tissue of foliate tassels, the intercellular spaces surrounding the vascular sheath cells, especially of the secondary vascular bundles, is the primary center of mycelial development. The sheath and later the phloem cells are pushed aside as individual mycelial strands enlarge to form large globular protoplasmic masses, encased in a thin wall that later may become quite thickened. Oospores are formed in close association with the vascular bundles. The xylem vessels become much enlarged, distorted, and closely appressed to the developing oospores. There is a pronounced development of the spiral thickenings of the vascular elements. Host cells surrounding developing oospores become thick walled. Leaf, sheath, and husk tissues are alternately thickened to as much as twice normal size, causing the outer surfaces to be macroscopically roughened and warty because of increased cell and vascular development. This distorting development of the parasite caused little or no deleterious staining reaction on host cells. The presence of a thick double spore-wall, spore-wall markings, and arrangement of relatively large nuclei of the multinucleate oospore closely follow those figured by McDonough for *Sclerophthora macrospora* of wheat.

Nutrition and metabolism of the tobacco wilt Fusarium. WOLF, FREDERICK T. *Fusarium oxysporum* var. *nicotianae* can grow in a wide variety of synthetic media. Maltose and xylose gave rise to greater growth than other carbon sources tested. Levulose, however, is converted into cell material with greater efficiency than is any other sugar. The fungus utilizes nitrate and ammonium nitrogen, but far greater growth was obtained in media containing aspartic acid, glutamic acid, or β-alanine as the nitrogen source. The growth obtained on media containing certain nucleic acid derivatives as nitrogen sources was not comparable to that obtained with the best of the amino acids. In shake cultures, pronounced morphogenetic changes occur, and growth of the fungus is yeast-like. Alcohol is the major end product of carbohydrate dissimilation in stationary cultures. Under favorable conditions, the organism produces a red water-soluble pigment, which has been identified as the xanthone, rubrofusarin. Attempts to identify the material responsible for wilting of diseased plants have been unsuccessful. Fusarinic acid, which has been implicated in the production of wilting by other Fusaria, is not formed by this organism.

Effect of zineb (Dithane Z-78) on the survival of cotton seedlings. YOUNG, D. W., AND G. A. BRANDES. Seedling diseases of cotton are very destructive in many areas of the United States. Because of the reduction in stand, the total annual loss from these diseases averages 500,000 bales of cotton per year. On examination of 545 infected seedlings, the following organisms were isolated with the indicated frequency: Rhizoctonia (413), Fusarium (243), Pythium (88), Rhizopus (23), Verticillium (12), Alternaria (7), and Penicillium (1). Data were also obtained from 48 out of 64 field tests conducted throughout the cotton belt in 1954. In these tests zineb (Dithane Z-78), which is zinc ethylenebis[dithiocarbamate], was suspended in 5 to 10 gal. of water and applied at 3.25 lb. active ingredient per acre by spraying into the seed furrow during planting time. Seedling counts showed increases in the stand ranging from 0 to 156 per cent with an over-all average increase of 19 per cent. Data also showed this treatment gave a 21 per cent decrease in the number of 6-in. spaces with no healthy cotton plants. In 9 tests in California, yields were increased by the zineb treatment an average of 207 lb. of lint per acre or 16 per cent. The stand increase in these tests was 19 per cent. No phytotoxic effects resulted from use of zineb treatments.

TRIARCH PRODUCTS

FINE MICROSCOPE SLIDES FOR DISCRIMINATING BIOLOGISTS

As we prepare and sell only microscope slides, we are able to give you better quality, better service and better prices than you can get elsewhere.

Remember, TRIARCH means ACCURACY, DEPENDABILITY, SERVICE

Write for our illustrated catalog No. 10

GEO. H. CONANT, RIPON, WISCONSIN

New fungicide treatment protects Potato Seed Pieces!

PHYGON-XL

controls rot up to 30 days

PHYGON-XL is one of the most economical and effective fungicides for destroying the fungi which attack unprotected seed potatoes in the ground. Phygon gives you better stands, higher yields, higher quality. Seed pieces won't rot up to 30 days after treatment, hence planting can be delayed in case of rain.

Phygon-XL® is today's cheapest, most effective organic fungicide for the control of fungus diseases on fruit trees, row crops and ornamentals.

Naugatuck Chemical

Division of United States Rubber Company

Naugatuck, Connecticut

US RUBBER

producers of seed protectants, fungicides, miticides, insecticides, growth retardants, herbicides: Spergon, Phygon, Aramite, Synklor, MH, Alanap, Duraset.

A Department of Plant Pathology will be established in the University of Illinois, College of Agriculture, for the beginning of the 1955-56 school year. Since 1942, graduate teaching in plant pathology has been administered by an interdepartmental committee and degrees have been granted but the system was somewhat cumbersome to administer. With the final approval by the Board of Trustees, pathologists now in the Departments of Agronomy, Botany, and Horticulture will be brought together as an autonomous group and will be directly responsible for all activities in plant pathology in the University and the Experiment Station.

The Elvin Charles Stakman Endowment Fund was established by friends and students to honor E. C. Stakman, Professor Emeritus at the University of Minnesota, for his lifetime contributions and his dedication to scholarly leadership in biology and agriculture, education and the humanities. A medal, scroll, and nominal monetary grant will be awarded for outstanding research in cereal pathology to an individual of any country. Nominations may be sent to the Department of Plant Pathology, University of Minnesota, St. Paul, on or before December 1 of each year and should include the nature and significance of the nominee's work, publications, and supporting letters from two colleagues.

Dedication ceremonies were held March 8 for the newly constructed group of buildings for Plant Pathology on the campus of South Dakota State College, Brookings. The new facilities will be used for both teaching and research and they consist of an office and laboratory building, three greenhouses 30 x 112 feet each, one smaller refrigerated greenhouse for summer use and a headhouse or service building. All buildings are connected by a central glassed-in corridor. In addition to classrooms and laboratories, the new buildings contain temperature controlled chambers, temperature, light, and humidity controlled inoculation rooms, cold storage rooms and temperature and photo-period controlled greenhouses. Among those present at the dedication ceremonies were: Dr. J. J. Christensen, University of Minnesota; Drs. W. F. Buchholtz, C. S. Reddy, J. R. Wallin, and A. L. Bakke, Iowa State College; Dr. W. G. Hoyman, North Dakota State College; and D. J. Fitchett, University of Nebraska.

The Gordon Research Conferences under the sponsorship of the AAAS will present two programs of interest to phytopathologists. They are: "Microbiological Deterioration" under the co-chairmanship of R. E. Vicklind and James G. Horsfall, from August 1-5 at the New Hampton School, New Hampton, N. H. The session will open on August 1 with a talk by R. H. Wellman on "Opportunities for progress in the prevention of microbiological deterioration" and a symposium on the chelation mechanism of fungicides under the chairmanship of Martin Rubin. August 2 will be devoted to a discussion on the organic sulfur fungicides under the chairmanship of R. A. Ludwig with Dr. van der Kirk of Holland as the guest conference speaker participating. August 3 will be devoted to a discussion of other fungitoxic mechanisms under the chairmanship of J. G. Horsfall; and Auugst 4 on the service life of fungicidal treatments under the chairmanship of R. F. H. Siu, and methodology and development of fungicides under the chairmanship of J. M. Leonard. The other conference of interest to botanists and plant pathologists will be held at Kimball Union Academy, Meridan, N. H. on July 25-29 on "Plant Biochemistry and Agriculture" under the chairmanship of J. van Overbeek and A. S. Crafts. The biochemistry of soils will be discussed on July 25 under the chairmanship of R. H. Burris; the biochemistry of plant nutrition on July 26 under the chairmanship of G. L. McNew; Biochemistry and physiology of plant growth on July 28 under the chairmanship of J. van Overbeek; and the energy transfer in plants on July 29 under the chairmanship of B. Axelrod. Further details on the speakers and subjects can be found in SCIENCE 121: 576 and 579 (April 22, 1955).

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

THE COST PER UNIT

The efficient use of fertilizer to lower unit cost of production is sound economics for the farmer at any time. It becomes doubly important in a situation of declining farm prices. Continuing research on all of the factors involved in the use of plant food to lower unit costs of food and feed production should do much to strengthen Agriculture's position in our National economy.

AMERICAN POTASH INSTITUTE, INC.

1102 Sixteenth St., N. W. **Washington 6, D. C.**

Member Companies: American Potash & Chemical Corporation • Duval Sulphur & Potash Company • Potash Company of America • Southwest Potash Corporation • United States Potash Company